DOI: 10.1002/adsc.200700303

# Supported Ionic Liquid Enzymatic Catalysis for the Production of Biodiesel

Muriell Gamba,[a] Alexandre A. M. Lapis,[a] and Jairton Dupont[a,*]

[a] Laboratory of Molecular Catalysis, Institute of Chemistry, UFRGS, Av. Bento Gonçalves, 9500 Porto Alegre 91501-970 RS, Brazil
Fax: (+55)-51-3308-7304; e-mail: dupont@iq.ufrgs.br

Received: June 22, 2007; Revised: September 12, 2007; Published online: December 14, 2007

Supporting information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

**Abstract:** *Pseudomonas cepacia* lipase supported in the 1-*n*-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ionic liquid is an alternative "green" method for the production of biodiesel from the alcoholysis of soybean oil. The transesterification reaction catalyzed by this ionic liquid-supported enzyme can be performed at room temperature, in the presence of water and without the use of organic solvents. It is also compatible with various alcohols (including isoamyl alcohol). The biodiesel is separated by simple decantation and the recovered ionic liquid/enzyme catalytic system can be re-used at least four times without loss of catalytic activity and selectivity.

**Keywords:** biodiesel; enzymes; ionic liquids; lipase

## Introduction

Renewable energy sources are in the center of concern of policy makers in view of economic and geopolitical factors such as high oil prices, environmental problems,[1] and supply instability.[2] Biodiesel (monoalcohol fatty acid esters) produced by alcoholysis of vegetable oils or animal fats, for example, becomes a very attractive alternative for diesel engines due to its similarity with petroleum-based fuel. The alcoholysis (in particular methanolysis) of vegetable oils catalyzed by metal hydroxides (or alkoxides) and sulfuric acid is the mostly used method for the generation of biodiesel. However, technological problems such as corrosion and emulsification are usually associated with these acid/base methodologies.[3–7] In this respect, there are huge scientific and technological efforts to minimize these problems by the development of heterogeneous catalysts,[8,9] organic bases, methanolysis under supercritical conditions[10] and enzymes.[11,12] Although several improvements have been introduced in the transesterification reaction (Scheme 1) such as, for example, the advent of new solid catalysts,[8] major problems like the use of solvents, separation of glycerol (main by-product), yield and regeneration of catalysts are still unsolved.


**Scheme 1.** Transesterification reaction of vegetable oils.

It is evident that the equilibrium to the biodiesel product can be increased by removal of the glycerol by-product.[13] In this respect, enzymatic transesterification using lipase has become very attractive for biodiesel fuel production.[14] The glycerol by-product can easily be recovered and the purification of fatty methyl esters is simple to accomplish. However, the main hurdle to the commercialization of this system is the cost of lipase production since the recovery of the enzyme is complicated. It has been reported several times that ionic liquids[15] are the support of choice for various enzymes. The association of enzymes with imidazolium ionic liquids usually generates more stable and active catalysts.[16,17] The enzyme/ionic liquid/stabilizer combination usually exhibits an excellent synergistic effect that enhances the activity and durability of the catalytic system. Moreover, in these ionic liquid multiphase processes, primary products can be extracted during the reaction to modulate the product selectivity (playing with the solubility of different substrates and reaction products in the catalyst-containing phase). This approach can promise a suitable method to avoid consecutive reactions of primary products and it has been exploited to some extent in ionic liquid biphasic catalytic processes.[18]

We disclose herein that the combination of 1-*n*-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)-imide ionic liquid (BMI·$NTf_2$) with lipases is an outstanding catalytic system for the generation of biodiesel. Indeed, the transesterification reaction catalyzed by this ionic liquid-supported enzyme can be performed at room temperature, in the presence of water, without the use of organic solvents and can be employed with various alcohols (including isoamyl alcohol). Furthermore, the biodiesel is separated by simple decantation and the recovered ionic liquid/enzyme catalytic system can be re-used at least four times without loss of its catalytic activity and selectivity.

## Results and Discussion

Initially we have examined the transesterification of soybean oil by varying the reaction temperature (20–60 °C), the amount of reagents, the ionic liquids (BMI·$BF_4$, BMI·$PF_6$ and BMI·$NTf_2$, Figure 1) and enzymes (*Candida rugosa*, *Pseudomonas cepacia*, *Rhizophus niveus*, *Pseudomonas fluorescens*, *Penicillium camembertii*, *Aspergillus niger*, *Penicillium roqueforti*, *Rhizophus niveus*, *Candida antarctica*, lipase from porcine pancreas, and *Mucor miehei*) with methanol (Table 1).

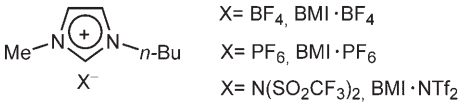



**Figure 1.** Imidazolium ionic liquids employed as the enzyme support.

*Pseudomonas cepacia* lipase was found to be the most effective enzyme and the use of BMI·$NTf_2$ in a ratio of 5/1 ionic liquid/commercial methanol gave the best vegetable oil conversions. The best reaction conversion (74 %) was obtained in 48 h and determined by thin layer chromatography[11] and by HPLC[19] analysis in triplicate experiments (Table 1, entry 5).

The reactions performed using the BMI·$BF_4$ and BMI·$PF_6$ gave similar conversions to that obtained in BMI·$NTf_2$. However, BMI·$NTf_2$ is the best choice since BMI·$BF_4$ is hydrophilic[20] thus rending more difficult the glycerol separation and BMI·$PF_6$ may hydrolyze and generate HF.[21]

**Table 1.** Products and conversions (by HPLC[19]) obtained from the transesterification reaction of soybean oil by lipases with methanol in BMI·$NTf_2$ at room temperature.

| Entry | *t* [h] | Lipase | Acids [%][a] | Mono [%][a] | Di [%][a] | Tri [%][a] | Biodiesel [%][b] |
|---|---|---|---|---|---|---|---|
| 1 | 24 | *Pseudomonas fluorescens* | 0 | 4.1 | 14.1 | 64.0 | 17.8 |
| 2 | 48 | *Pseudomonas fluorescens* | 0 | 7.1 | 16.5 | 49.4 | 27.0 |
| 3 | 24 | *Pseudomonas cepacia*[c] | 2.0 | 16.3 | 14.0 | 43.0 | 24.7 |
| 4 | 48 | *Pseudomonas cepacia*[c] | 4.0 | 38.7 | 8.5 | 0 | 48.8 |
| 5 | 24 | *Pseudomonas cepacia*[d] | 0.1 | 13.4 | 7.6 | 5.0 | 73.9 |
| 6 | 48 | *Pseudomonas cepacia*[d] | 0 | 11.4 | 8.0 | 6.0 | 74.6 |
| 7 | 24 | *Pseudomonas cepacia* | 0.6 | 3.6 | 6.9 | 67.9 | 21.2 |
| 8 | 48 | *Pseudomonas cepacia*[e] | 0.5 | 5.8 | 6.2 | 48.4 | 39.1 |
| 9 | 24 | *Candida rugosa*[f] | 0 | 0.7 | 3.2 | 96.1 | 0 |
| 10 | 24 | *Penicillium camembertii*[g] | 0.4 | 0 | 6.3 | 92.6 | 0.7 |
| 11 | 24 | *Aspergillus niger*[h] | 0 | 0 | 7.9 | 92.1 | 0 |
| 12 | 24 | *Penicillium roqueforti*[i] | 0 | 0.6 | 7.7 | 91.7 | 0 |
| 13 | 24 | *Rhizophus niveus* | 0 | 0 | 3.2 | 97.8 | 0 |
| 14 | 24 | *Candida antarctica*[j] | 0.6 | 0.6 | 4.1 | 56.1 | 38.6 |
| 15 | 24 | *Porcine pancreas*[k] | 4.7 | 0 | 8.3 | 84.7 | 2.3 |
| 16 | 24 | *Mucor miehei*[l] | 0.2 | 0 | 7.0 | 90.2 | 2.6 |

[a] Fatty acids, mono-, di- and tri-glycerides.
[b] % of conversion of biodiesel (v %) as previously described in ref.[19]
[c] PS-D Amano I.
[d] PS-C Amano I.
[e] PS-Amano.
[f] Amano AY.
[g] Amano G 50.
[h] Amano A.
[i] R Amano K.
[j] Novozyme 435.
[k] Lipase from *Porcine pancreas* (type II, Aldrich).
[l] Lipozyme IM.

**Table 2.** Products and conversions (by HPLC[19]) obtained from the transesterification reaction of soybean oil by *Pseudomonas cepacia* lipase (PS-Amano) with alcohols in BMI·$NTf_2$ at room temperature.

| Entry | Alcohol | *t* (h) | Acids [%][e] | Mono [%][f] | Di [%][f] | Tri [%][f] | Biodiesel [%][g] |
|---|---|---|---|---|---|---|---|
| 1 | MeOH[a] | 6 | 6.8 | 42.7 | 9.0 | 0 | 41.4 |
| 2 | MeOH[a] | 24 | 6.0 | 7.7 | 0 | 0 | 86.2 |
| 3 | MeOH[a] | 48 | 3.7 | 0 | 0 | 0 | 96.3 |
| 4 | MeOH[b] | 6 | 0 | 2.8 | 7.5 | 67.2 | 22.4 |
| 5 | MeOH[b] | 24 | 2.9 | 8.8 | 4.1 | 0 | 84.1 |
| 6 | MeOH[b] | 30 | 0.5 | 3.4 | 4.0 | 0 | 92.1 |
| 7 | MeOH[c] | 6 | 0 | 0 | 4.3 | 92.8 | 2.8 |
| 8 | MeOH[c] | 24 | 0.6 | 3.5 | 6.9 | 67.8 | 21.1 |
| 9 | MeOH[c] | 72 | 0.4 | 8.7 | 6.1 | 34.2 | 50.5 |
| 10 | EtOH[d] | 6 | 1.6 | 27.2 | 8.9 | 0 | 62.2 |
| 11 | EtOH[d] | 24 | 10.6 | 1.4 | 4.2 | 0 | 83.7 |
| 12 | EtOH[d] | 30 | 3.7 | 0 | 0 | 0 | 96.3 |
| 13 | EtOH[c] | 6 | 0 | 1.5 | 7.8 | 81.8 | 8.7 |
| 14 | EtOH[c] | 24 | 0.8 | 10.4 | 14.2 | 46.5 | 28.0 |

[a] MeOH 70%.
[b] MeOH 90%.
[c] MeOH or EtOH 100%.
[d] EtOH 85%.
[e] Fatty acids.
[f] Mono-, di- and tri-glycerides.
[g] % of conversion of biodiesel (v%) as previously described in ref.[19]

Additional studies verified that the reaction rate is strongly influenced by the water content in the alcohols. The reactions performed in the absence of water furnish lower reaction rates than those performed with hydrated alcohols (Table 2 and Figure 2).

The addition of water up to 5 v% is necessary to improve the reaction rate. 30 v% water addition gave the best results for methanol (96% conversion, 48 h) and 15 v% water addition for ethanol (96% conversion, 30 h) see Table 2 and Figure 2.

Interestingly, the presence of water improves the oil hydrolysis rate yielding the fatty acid that is converted into the respective ester faster than the transesterification pathway (Scheme 2).

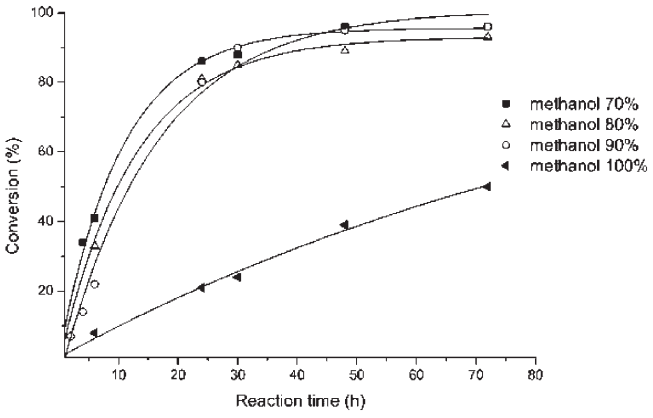
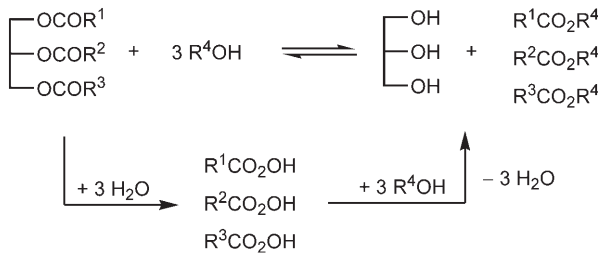


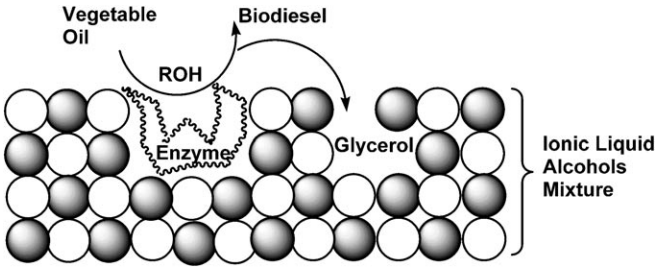

**Figure 2.** Conversion of soybean oil to biodiesel using methanol with different water contents by *Pseudomonas cepacia* lipase (PS-Amano).

The best reaction conditions for biodiesel production were obtained by the use of *Pseudomonas cepacia* lipase (0.6 g) in BMI·$NTf_2$ (8.2 mmol) as a support, methanol/water (70:30) (41.2 mmol) and soybean oil (3.4 mmol) at room temperature (30 °C). The reaction time (48 h) for the complete conversion of the starting oil material was first determined by thin layer chromatography.[11] This result was corroborated afterwards by triplicate experiments in which the reaction conversion was determined as 96% by HPLC[19] (see Figure S1 in the Supporting Information). These catalytic activities are superior to those reported earlier for enzymatic processes in water or other supports.[11,22,23] This result is probably related to the extraction of the glycerol – formed during the transesterification – by the ionic liquid/alcohol mix-




**Scheme 2.** Hydrolysis, transesterification and esterification reaction catalyzed by enzymes supported in ionic liquids.




**Scheme 3.** Transesterification of vegetable oil catalyzed by enzymes supported in ionic liquids with concomitant capture of the glycerol by-product.

ture[24] thus shifting the equilibrium to the biodiesel product (Scheme 3).

Note that the solubility of glycerol in pure ionic liquid is very low (>1 w%); however it can reach 15 w% in the mixture of the ionic liquid with methanol (ratio 1/9 mol%). The relatively lower reaction rates may be attributed to the lower solubility of the triglyceride in the BMI·$NTf_2$ ionic liquid as already observed in the lipase-catalyzed glycerolysis[25] of triolein.[26] Therefore, it can envisaged that more efficient catalytic systems[27] may be obtained by the use of task-specific ionic liquids such as those containing hydrophobic substituents in the cation that are known to contribute to the increase of triglyceride solubility and consequently can cause an enhancement of the initial reaction rate.[26]

The catalytic systems can be used in scales of 30–50 grams and the biodiesel was obtained in a 92% isolated yield (97% purity) after 35 h for ethanol and with 90% yield (91% purity) for methanol after 24 h. The methyl and ethyl esters are easily purified by a simple decantation of the biphasic system and the ionic liquid/enzyme phase could be reused at least 4 times without any significant lost of catalytic activity and selectivity. After this period it is necessary to remove the enzyme from the ionic liquid by filtration and wash it with water to recover the catalyst that may be reincorporated into new reaction media. The ionic liquid that still contains the glycerol should be washed with water to separate the glycerol that is isolated with high purity (>98%). Note that the glycerol isolated from classical acid or basic catalysis needs various purification steps to achieve desirable purity. The recovered ionic liquid can be reused after drying under reduced pressure. In addition, the transesterification of soybean oil using the *Pseudomonas cepacia* lipase supported in the BMI·$NTf_2$ catalytic system can also be performed with isoamyl alcohol to afford biodiesel in 90% yield after 36 h at room temperature. This result indicates that the biodiesel may be produced with fusel oil that is a by-product of the distillation of ethyl alcohol from the fermentation of molasses and contains mainly $C_3$–$C_5$ alcohols.[28]

## Conclusions

In summary, we have demonstrated that *Pseudomonas cepacia* lipase supported in BMI·$NTf_2$ is an alternative "green" method for the production of biodiesel from the alcoholysis of soybean oil. The ionic liquid provides the ideal medium for the stabilization of the enzyme and also for the removal of glycerol by-product and for increasing the biodiesel yield. This method may also be very useful for other transesterification (esterification) reactions.

## Experimental Section

### General Remarks

Alcohols, enzymes and all other chemicals were purchased from commercial sources (Amano, Acros or Aldrich) and used without further purification. The ionic liquids were prepared as previously described.[29] The HPLC experiments were performed on a Shimadzu LC-20A Prominence liquid chromatograph equipped with an SPD-M20A diode-array detector and a four-solvent delivery system.

The solvents were filtered through a 0.45 μm Millipore filter prior use and degassed by continuous stripping with nitrogen. Injection volumes of 10 μL and a flow rate of 1 mL $min^{-1}$ were used in all experiments. All samples were dissolved in 2-propanol-hexane (5:4, v/v). All solvents were of HPLC grade and were used as obtained, without further purification. A column, Shim-pack VP-ODS (particle size 4.60 μm, 250×4.6 mm I.D.), was obtained from Shimadzu. HPLC method: reservoir A contained water, reservoir B contained acetonitrile and reservoir C contained 2-propanol-hexane (5:4, v/v). A 50 min ternary gradient with two linear gradient steps was employed: 30% A+70% B in 0 min, 100% B in 15 min, 50% B+50% C in 30 min, followed by isocratic elution with 50% B+50% C for the last 20 min.[19] The HPLC data of the enzymatic reactions are available in the Supporting Information.

### General Procedure for Biodiesel Production

In a 250-mL round-bottom flask, lipase (*Pseudomonas cepacia*) (6 g) was supported in BMI·$NTf_2$ (24 mL, 81.9 mmol). To the supported catalyst was added soybean oil (30 g, 34.3 mmol) and methanol (16.5 mL, 411.8 mmol) and a biphasic system was observed. The reaction media was stirred for 24 h (monitored by HPLC). After decantation, the phase containing the biodiesel was removed and filtered to remove some enzyme particles. The biodiesel was obtained in 95% yield (96% of conversion by HPLC). The catalyst could be reused at least four times.

## Acknowledgements

*Thanks are due to CAPES and CNPq for partial financial support and to NOVOZYMES LATIN AMERICA (Araucaria PR, Brazil) for a sample of Novozyme 435.*

## References


[1] D. Tilman, J. Hill, C. Lehman, *Science* **2006**, *314*, 1598–1600.
[2] M. Frondel, J. Peters, *Energy Policy* **2007**, *35*, 1675–1684.
[3] Y. J. Liu, E. Lotero, J. G. Goodwin, C. Q. Lu, *J. Catal.* **2007**, *246*, 428–433.
[4] S. M. P. Meneghetti, M. R. Meneghetti, C. R. Wolf, E. C. Silva, G. E. S. Lima, L. D. Silva, T. M. Serra, F. Cauduro, L. G. de Oliveira, *Energy Fuels* **2006**, *20*, 2262–2265.
[5] F. Chai, F. Cao, F. Zhai, Y. Chen, X. Wang, Z. Sua, *Adv. Synth. Catal.* **2007**, *349*, 1057–1065.
[6] F. R. Abreu, M. B. Alves, C. C. S. Macedo, L. F. Zara, P. A. Z. Suarez, *J. Mol. Catal. A: Chem.* **2005**, *227*, 263–267.
[7] F. R. Abreu, D. G. Lima, E. H. Hamu, S. Einloft, J. C. Rubim, P. A. Z. Suarez, *J. Am. Oil Chem. Soc.* **2003**, *80*, 601–604.
[8] M. Toda, A. Takagaki, M. Okamura, J. N. Kondo, S. Hayashi, K. Domen, M. Hara, *Nature* **2005**, *438*, 178–178.
[9] B. A. DaSilveira Neto, M. B. Alves, A. A. M. Lapis, F. M. Nachtigall, M. N. Eberlin, J. Dupont, P. A. Z. Suarez, *J. Catal.* **2007**, *249*, 154–161.
[10] H. Y. He, T. Wang, S. L. Zhu, *Fuel* **2007**, *86*, 442–447.
[11] H. Fukuda, A. Kondo, H. Noda, *J. Biosci. Bioeng.* **2001**, *92*, 405–416.
[12] W. Du, L. Wang, D. H. Liu, *Green Chem.* **2007**, *9*, 173–176.
[13] J. M. Marchetti, V. U. Miguel, A. F. Errazu, *Ren. Sust. Energ. Rev.* **2007**, *11*, 1300–1311.
[14] S. Al-Zuhair, *Biotechnol. Prog.* **2005**, *21*, 1442–1448.
[15] J. Dupont, P. A. Z. Suarez, *Phys. Chem. Chem. Phys.* **2006**, *8*, 2441–2452.
[16] T. De Diego, P. Lozano, S. Gmouh, M. Vaultier, J. L. Iborra, *Biomacromol.* **2005**, *6*, 1457–1464.
[17] P. Lozano, E. García-Verdugo, R. Piamtongkam, N. Karbass, T. De Diego, M. I. Burguete, S. V. Luis, J. L. Iborra, *Adv. Synth. Catal.* **2007**, *349*, 1077–1084.
[18] E. T. Silveira, A. P. Umpierre, L. M. Rossi, G. Machado, J. Morais, G. V. Soares, I. L. R. Baumvol, S. R. Teixeira, P. F. P. Fichtner, J. Dupont, *Chem. Eur. J.* **2004**, *10*, 3734–3740.
[19] M. Holcapek, P. Jandera, J. Fischer, B. Prokes, *J. Chromatogr. A* **1999**, *858*, 13–31.
[20] P. A. Z. Suarez, S. Einloft, J. E. L. Dullius, R. F. de Souza, J. Dupont, *J. Chim. Phys. Phys.-Chim. Biol.* **1998**, *95*, 1626–1639.
[21] G. S. Fonseca, A. P. Umpierre, P. F. P. Fichtner, S. R. Teixeira, J. Dupont, *Chem. Eur. J.* **2003**, *9*, 3263–3269.
[22] Y. Watanabe, Y. Shimada, A. Sugihara, Y. Tominaga, *J. Mol. Catal. B: Enz.* **2002**, *17*, 151–155.
[23] Y. Shimada, Y. Watanabe, A. Sugihara, Y. Tominaga, *J. Mol. Catal. B: Enz.* **2002**, *17*, 133–142.
[24] A. P. Abbott, M. P. M. Cullis, M. J. J. Gibson, R. C. C. Harris, E. Raven, *Green Chem.* **2007**, *8*, 868–872.
[25] Z. Guo, B. Q. Chen, R. L. Murillo, T. W. Tan, X. B. Xu, *Org. Biomol. Chem.* **2006**, *4*, 2772–2776.
[26] B. Chen, Z. Guo, T. Tan, X. Xu, *Biotechnol. Bioeng.* **2007**, doi: 10.1002/bit. 21520.
[27] J. B. Domingos, J. Dupont, *Catal. Commun.* **2007**, *8*, 1383–1385.
[28] H. J. Rehm, G. Reed, A. Puhler, P. Stadler, H. Sahm, *Biotechnology: A Multi-Volume Comprehensive Treatise: Biological Fundamentals*, 2 edn., John Wiley & Sons, New York, **1991**.
[29] C. C. Cassol, G. Ebeling, B. Ferrera, J. Dupont, *Adv. Synth. Catal.* **2006**, *348*, 243–248.