Quim. Nova, Vol. 38, No. 3, 303-308, 2015 http://dx.doi.org/10.5935/0100-4042.20150031

Artigo

MICROWAVE ACTIVATION OF IMMOBILIZED LIPASE FOR TRANSESTERIFICATION OF VEGETABLE OILS

Monna Lisa B. Queiroz[a], Rachel Freire Boaventura[a], Micael Nunes Melo[a], Heiddy M. Alvarez[b], Cleide M. F. Soares[a], Álvaro S. Lima[a], Montserrat F. Heredia[a], Cláudio Dariva[a] and Alini T. Fricks[a,*]

[a]Instituto de Tecnologia e Pesquisa, Universidade Tiradentes, Av. Murilo Dantas, 300, 49032-490 Aracaju – SE, Brasil

[b]Departamento de Ciências Exatas, Universidade Estadual de Feira de Santana, km 03 BR 116, 44031-460 Feira de Santana – BA, Brasil

Recebido em 04/06/2014; aceito em 03/12/2014; publicado na web em 05/03/2015

This work investigated the effect of microwave irradiation (MW) on the ethanolysis rate of soybean and sunflower oils catalyzed by supported Novozyme 435 (*Candida antarctica*). The effects of *tert*-butanol, water addition and oil:ethanol molar ratio on transesterification were evaluated under conventional heating (CH), and under optimum reaction conditions (with no added water in the system, 10% tert-butanol and 3:1 ethanol-to-oil molar ratio). The reactions were monitored up to 24 h to determine the conditions of initial reaction velocity. The investigated variables under MW (50 W) were: reaction time (5.0-180 min) and mode of reactor operation (fixed power, dynamic and cycles) in the absence and presence of tert-butanol (10% (w/w). The measured response was the reaction conversion in ethyl esters, which was linked to the enzyme catalytic activity. The results indicated that the use of microwave improved the activity at fixed power mode. A positive effect of the association of *tert*-butanol and MW irradiation on the catalytic activity was observed. The reaction rate improved in the order of approximately 1.5 fold compared to that under CH with soybean oil. Using soybean oil, the enzymatic transesterification under MW for conversion to FAEE (fatty acid ethyl esters) reached >99% in 3h, while with the use of CH the conversions were about 57% under similar conditions.

Keywords: lipase; microwave; FAEE; biodiesel.

INTRODUCTION

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are used as versatile biocatalysts in modern organic chemistry, particularly for modification of fats and other lipids via hydrolysis, esterification and interesterification.[1,2] Biotransformations catalyzed by lipases can be conducted at room temperature and normal pressure, as compared with the high energy costs of conventional chemical processes.[3]

Biodiesel is an important biodegradable renewable fuel for alternative diesel engines.[4,5] In this field, considerable attention has been devoted in the last decade for the synthesis of fatty acid ethyl and methyl esters (FAEE and FAME, respectively) using lipases as catalysts,[6-9] as it is a cleaner process, yields a glycerol free of salt contaminants, and the processes does not require a neutralization step, common drawbacks inherent in the acid or base catalyzed process.[10] Traditionally, the transesterification reaction is carried out mixing an excess of an alcohol. Despite the high reactivity of methanol in this reaction the use of ethanol is a promising alternative due to its lower toxicity and the higher yield on a weight basis.[11] It should also be considered the ethanol's renewable nature, and that fact the in Brazil, ethanol is widely and prompt available.[12] Additionally, the replacement of methanol with less polar alcohols seems to result in a slight increase in the retained activity. On the other hand, a significant inactivation of lipase can occurs.[13,14]

Despite the obvious advantages in terms of reduction in energy costs and better quality of biodiesel obtained by the enzymatic process, the main problem with lipase catalytic biodiesel production is the low reaction rate, making necessary to develop methods to increase the reaction rate. In this sense, microwave irradiation has became a proven tool for accelerating organic synthesis with dramatic increase of reaction[15,16] and emulsion separation[17] rates. Microwave irradiation offers a clean, inexpensive, and convenient method of heating, to introducing energy into chemical systems. Microwave effects in chemical reactions are related to the short range molecular motion due continuous polar molecules (or dipoles) order to the electric fields caused by microwave radiation. This process of ordered dipoles (with electromagnetic field) and disordered (without the field), enhances the molecular attrition increasing the local temperature and reaction rates.[18,19]

Applications of microwave irradiation technology for enzymatic synthesis have been developed in the last years. Many reactions catalyzed by lipases have provided good results when performed under microwave irradiation, including: racemization and kinetic resolution of chiral amines and secondary alcohols,[20,21] acylation,[22] hydrolysis of vegetable oils,[23,24] esterification,[25,26] and transesterification.[27,28] Loupy and co-workers have studied the effectiveness of microwave irradiation in increasing the enzymatic affinity and selectivity of supported lipases in esterification and transesterification reactions under dry media conditions.[29]

In particular to enzymatic transesterification of vegetables oils using microwave irradiation for biodiesel production, most of the works in the literature use methanol as the acyl acceptor[12,30] and also few efforts are made about the modes of operation of the microwave reactor for better understanding of the difference of thermal and non thermal effects of microwave irradiation on lipase reaction rates.

The main objective of this work was to investigate the effect of microwave irradiation and operation mode of microwave on the rate of transesterification of soybean and sunflower oils with ethanol catalyzed by supported enzyme, Novozyme 435 (*Candida antarctica*) for synthesis of FAEE for biodiesel production (Figure 1). The reactions were conducted under two different conditions: microwave irradiation (MW) and conventional heating (CH). It was observed that the reaction rate was significantly increased when using microwave irradiation.

*e-mail: alinitf@yahoo.com.br

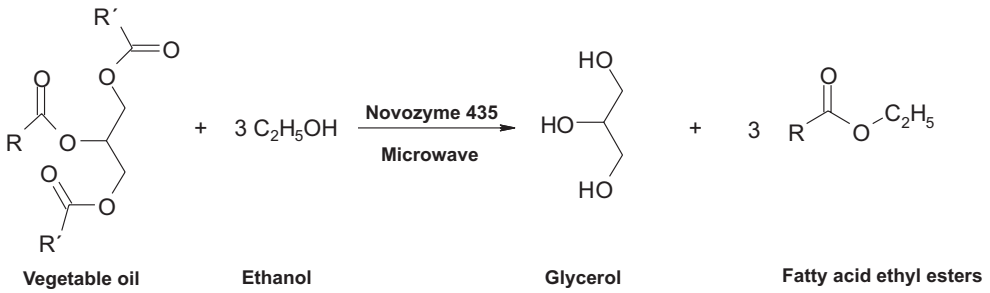

Figure 1. *Transesterification of soybean and sunflower oils and ethanol with Novozyme 435 under MW*

EXPERIMENTAL

Enzyme and chemicals

Commercial immobilized lipase *C. antarctica* (Novozyme 435) was kindly donated by Novozym®. The soybean and sunflower oils (10 ± 1 mg $KOH.g^{-1}$) were purchased in local market (Soya®, Brazil). The sunflower oil is of the 875.2 g mol^{-1} average molar mass having the following fatty acid distribution: 6.59% 16:0; 3.98% 18:0; 21.22% 18:1; 68.06% 18:2 and 0.15% 20:0 and soybean oil 871.9 g mol^{-1}: 11.3% 16:0; 3.5% 18:0; 23.6% 18:1; 54.7% 18:2; 6.9% 18:3.[14,31] Ethanol (> 99.5%), *n*-hexane, *n*-heptane (Vetec Química, RJ, Brazil) and methyl heptadecanoate (Aldrich, Milwaukee, WI, USA). All the other reagents were of analytical grade.

FAEE enzymatic synthesis

All the experiments (CH and MW) were performed in triplicate at 60 °C, using 1.5 g of vegetable oil (1.7 mmols of triglycerides) and ethanol as acyl acceptor. All reactants for the transesterification were previously dried before use. Blank tests were performed in the absence of enzyme. At the end of the reaction, the mixture was centrifuged for 2 min to remove the enzyme. The samples were washed with saturated solution of NaCl to remove the glycerol formed. The organic phase was extracted with *n*-hexane and treated with anhydrous Na_2SO_4. After evaporation of the solvent the sample was subjected to chromatographic analysis (GC).

Transesterification using conventional heating (CH)

Transesterification using CH was performed in a 10 mL hermetically sealed reactor immersed in a thermostatic bath with constant stirring at 150 rpm. Initially it was investigated the best condition for transesterification using CH, employing soybean oil, reaction time of 3 hours and 3.36% (w/w) of enzyme. The variables studied were: co-solvent (tert-butanol) addition (0-20.0% w/w), water addition (0 – 4.0% w/w), oil:ethanol molar ratio (1:1.5 – 1:18). All percentages were based on oil weight. *Tert*-butanol was chosen as co-solvent because is a non-toxic solvent of relative low cost.[32] Under the condition that promotes the best conversion (oil:ethanol molar ratio 1:3, tert-butanol 10% (w/w), without water addition) the formation of biodiesel from soybean and sunflower oils using CH was monitored up to 24 h to determine the conditions of the reaction initial velocity.

Transesterification using microwave irradiation (MW) heating

All experiments using MW heating were conducted in a microwave reactor (Model Discover SP, University-Wave, CEM Corporation), able to provide 300 W of continuous microwave power. Microwave reactor consists of a cylindrical internal chamber of 25 mm in diameter and 100 mm in height. The cylindrical design assures homogeneous irradiation over the whole working volume. This reactor can operate in distinct modes, named here by convenience of dynamic (D), power cycling (PC) and fixed power (FP) modes. In the dynamic method after selected the temperature set point, the system will bring the reaction mixture to that temperature as quickly and safely as possible and hold at the temperature control point for the hold time desired. In the power cycling mode input power, power internal, cooling interval, minimum and maximum temperature and the number of cycles is first adjusted. The system will bring the system to the maximum temperature; turn the power of to cool to the minimum temperature and then repeat this cycle for the user defined number of cycles. Fixed power mode enables to choose the input power level and irradiation time, where at that conditions the system irradiates the. In the present work, in any mode of operation, the reactor was operated with controlled temperature (60 °C) and power max "on" at 50 W of input power. An infrared sensor located in the bottom of the chamber monitored the reaction temperature. The temperature was controlled with compressed air blown into the chamber. This chilling air was designed to operate at different levels of pressure and either on continuous or intermittent flow mode.

The investigated variables under MW were: reaction time (22.0-180 min) at fixed power in the absence and presence of *tert*-butanol (10% (w/w)) and also the mode of reactor operation (dynamic and power cycling). Vegetable oils transesterification reactions were performed under dynamic mode (22 min) and power cycling mode (29, 44 and 88 cycles of 25 seconds each and cooling interval of 45, 30 and 15 seconds, respectively, which results in 34; 40 and 59 min of total reaction times, respectively). The results of catalytic activity in each operating mode were calculated under conditions of initial velocity and residual activity (RA) was obtained comparing to the CH in the presence of *tert*-butanol (residual activity, RA 100%).

Ethyl esters analysis

FAEE measurements of products were done by using the European standard test method of EN 14103 with minor modifications. FAEE contents in the reaction mixture have been quantified by gas chromatograph using a Shimadzu CG (CG-2010) with a cross bond carbowax (30 m × 0.32 mm ID × 0.25 μm film thickness) column. The column temperature was held at 170 °C for 1 min, then increased to 210 °C at 10 °C min^{-1}, and held at that degree for 1 min, increased again to 225 °C at 6 °C min^{-1}, held at that degree for 5.5 min. H_2 was used as a carrier gas at a pressure 41.9 kPa and flow rate of 1.5 mL min^{-1}. The injector was held at 250 °C with split flow rate 34.5 mL min^{-1}; and the FID detector at 250 °C. Total time of analysis: 14 min.

GC samples were prepared by dissolving 0.1 g final product on 10 mL of *n*-heptane. 100 μL of this solution and methyl heptadecanoate (2.5 mg mL^{-1}) as internal standard were transferred to a flask of 1 mL. 1 μL of this sample was then injected into a CG system in a

split mode (split ratio 1:20). The reaction conversion was calculated by taking into account the mass of ester content obtained by GC analysis and the total theoretical ester mass. All GC measurements were performed in duplicate.

Enzymatic activity

Conversion values obtained were connected to the catalytic activity: one unit (U) of enzyme specific activity was defined as the amount of enzyme that was necessary to produce 1 μmol FAEE per minute at conditions of initial velocity of reaction.

Enzymatic activities were calculated according to Equation 1.

$$A = (C * n/ 100) / t * m \quad (1)$$

where: A is the transesterification lipase activity (μmol min g^{-1} or U g^{-1}), C is conversion (%), n is number of μmols FAEE, in the case of 100% conversion (considering 875.2 and 871.9 g mol^{-1}average molar mass to sunflower and soybean oils, respectively), t is the reaction time in minutes (with system in initial condition of reaction) and m is mass of biocatalyst (g).

RESULTS AND DISCUSSION

Transesterification using CH: Determination of optimum conditions

Transesterification of vegetable oils is very sensitive to the reaction conditions (molar ratio ethanol:oil, water content and presence of organic solvent). The effect of variables: addition of co-solvent (*tert*-butanol), water addition and oil:ethanol molar ratio on the enzymatic conversion of soybean oil under CH are shown in Figure 2. The water in the reaction medium is known to strongly influence the reaction rate and this parameter analysis is sometimes optimized by the deliberate addition of water in the reaction medium.[33] In this study known amounts of water (0 - 4%) were added to the system in order to establish the best amount of water to be added to the system. FAEE yield seems to decrease with water content suggesting that *C. antarctica* lipase itself appears to contain sufficient water to preserve the catalytic conformation. The result obtained in the present work is in agreement with the fact that CAL-B (*C. antarctica lipase* free) does not require the presence of an oil/water interface to show higher activities. Tamalampudi and co-workers using the same lipase in their study have concluded that the rate of methanolysis decreases with the increase in the water content, reaching the FAME content of 75%, with no added water in the system.[34] Another research has also shown that CAL-B needs a nearly anhydrous reaction medium to be effective.[35] Additionally, the equilibrium of the reaction in the presence of water may become unfavorably which causes ester hydrolysis leading to lower conversions.

It is well known that *C. antarctica* lipase tolerates reaction media containing organic solvents, generally lipase has high synthesis activity and good stability observed in hydrophobic solvents like hexane,[36-38] but the hydrophilic compounds used as substrate (alcohol) or obtained as product (glycerol) are immiscible in hydrophobic reaction medium. Problems of solubilization result in the absorption of polar molecules onto the hydrophilic support leading to low transesterification rate.[38] To solve this problem, *tert*-butanol acts as an ideal solvent. *Tert*-butanol which is a moderately hydrophilic solvent (log P = 0.80) can solubilize oil, ethanol and glycerol, so the negative effect caused by methanol and glycerol on lipase catalytic activity could be totally eliminated.[39] In this study, *tert*-butanol solvent (10%) therefore improved the enzyme catalytic properties leading to higher

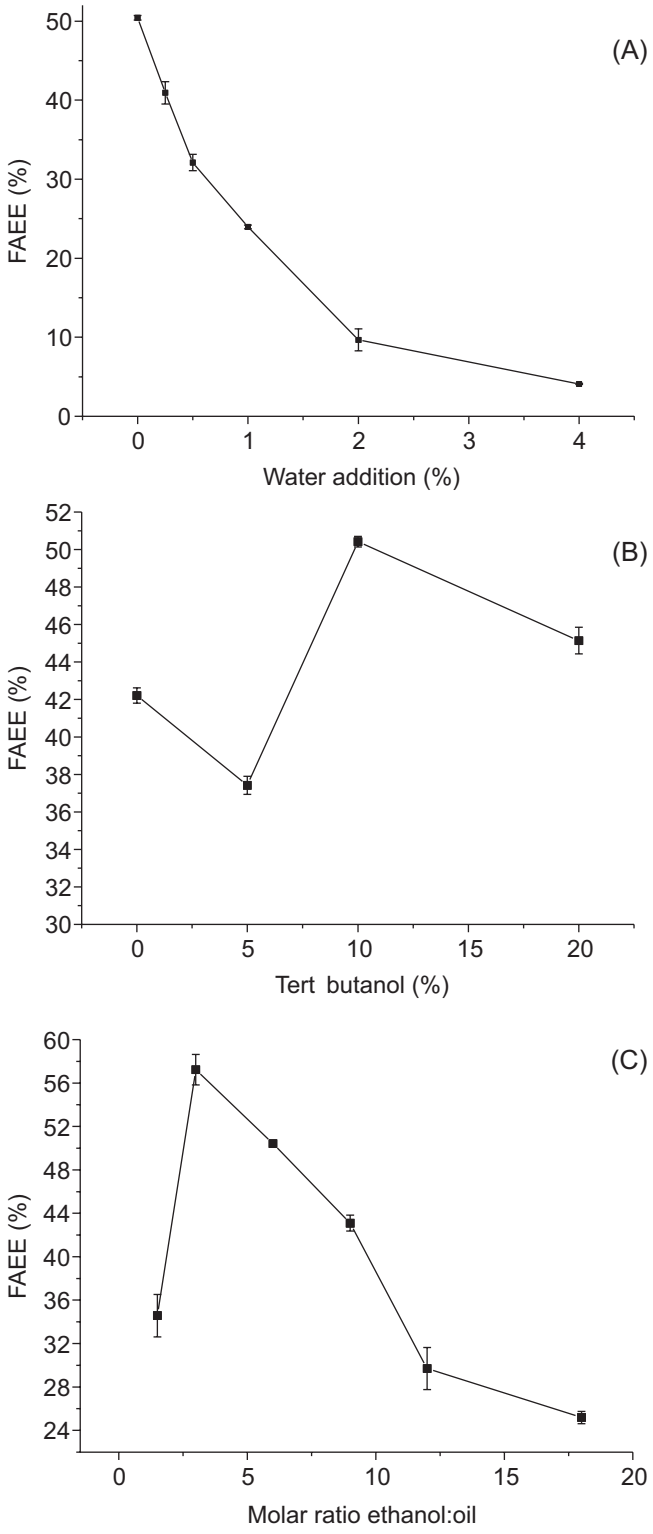

Figure 2. *Effect of water addition (A), tert-butanol (B) and molar ratio (C) on the transesterification of soybean oil catalyzed by Novozyme 435 under CH. Conditions: 60 °C, 3.36% enzyme loading*

FAEE yield (50.4), but higher concentrations of *tert*-butanol did not favor the activity of the enzyme in CH (Figure 2b).

With respect to the molar ratio alcohol:oil, the ratio is known to affect the reaction rate by altering the enzyme denaturation rate.[6] It appears that the yield of ethyl esters increases at low ethanol concentration (Figure 2c). The lowest yields can be expected at high ethanol:oil ratios. Alcohol in excess from the stoichiometric molar

ratio is reported to be required to ensure higher reaction rates but, due to its low solubility in triglycerides, undissolved ethanol also inhibits the enzymatic ethanolysis at higher molar ratios.[40]

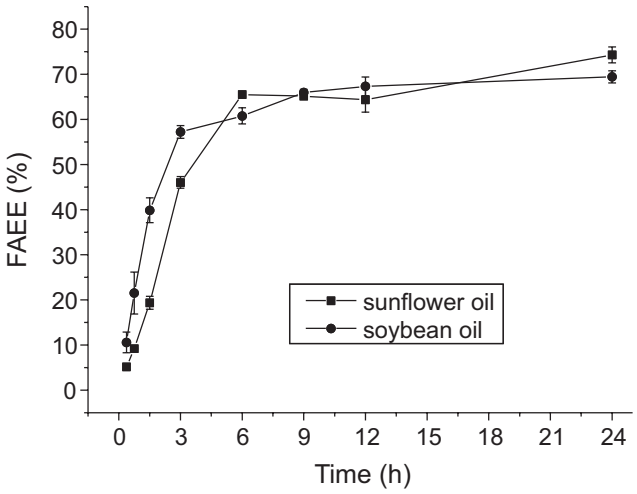

Figure 3. *Transesterification progress. Conditions: 60 °C, 1:3 oil-to-ethanol molar ratio, 3.36% enzyme loading, tert-butanol 10%, addition water 0%*

The optimum reaction conditions for the FAEE synthesis are selected to be the following: with no added water in the system, 10% *tert*-butanol and 3:1 ethanol to oil molar ratio. Under these conditions, the kinetics of formation of FAEE derived from soybean oil and sunflower were monitored up to 24 h to determine the conditions of initial velocity (Figure 3). The enzymatic transesterification using CH for both vegetable oils occurs at a constant rate until the reaction time of 1.5 h (90 min), in this reaction time was observed proportionality between the reaction time and conversion obtained. Thus, this reaction time (90 min) was considered for calculation of enzymatic activity of transesterification of vegetable oils. The activities found were 454 and 221 U g^{-1} using soybean oil and sunflower respectively. These values were considered 100% activity, and were used to calculate the residual activity of Novozyme 435 in the microwave experiments. These data show that the kinetics of transesterification of sunflower oil catalyzed by Novozyme 435 is slower compared to soybean oil. Also, for both oils the system tends to equilibrium after 6 hours of reaction.

Comparison microwave versus conventional heating systems

The heating of liquids using microwaves can be explained by the interaction of matter with the electromagnetic field of the incident radiation, causing the movement of ions as well as that of induced or permanent dipoles. Besides the quick heating of the materials, some authors consider that the microwaves can provide specific effects (not purely thermal) generally connected to the selective absorption of microwave energy by polar molecules.[41] According to this concept, microwave irradiation results in a change of thermodynamic properties of the reactive systems. An example of this effect would be the reduction of Gibbs free energy of activation of reactions to be due to the storage of microwave energy as energy vibration of a molecule or functional group (enthalpy effect), or by the alignment of the molecules (entropic effect).[42] In addition, it is believed that microwaves favor the efficiency of collisions molecular, due to the orientation of polar molecules involved in the reaction. All these properties make the microwave irradiation an interesting tool for the enzymatic process.

Figure 4 shows the results of transesterification of vegetable oils using MW heating with and without tert-butanol (mode of reactor operation: fixed power – FP) and using CH with tert-butanol, up to 180 minutes of reaction.

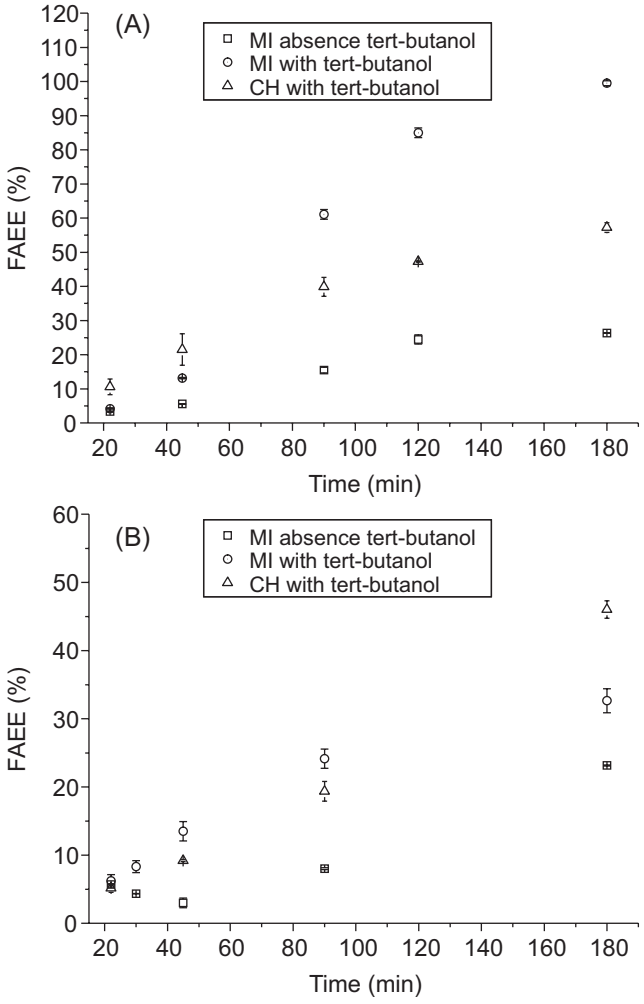

Figure 4. *Transesterification of soybean (A) and sunflower (B) oils catalyzed by Novozyme 435 under MW by fixed power (FP) mode (with and without tert-butanol) and CH (with tert-butanol). Conditions: 60 °C, 3.36% enzyme loading*

As observed the rate of enzymatic transesterification rate of soybean and sunflower oils were positively influenced by microwave irradiation in the presence of tert-butanol (10%). Using MW heating the conversions about 100% to soybean oil in 180 min, while using the CH the conversion was 57.2% (Figure 4A and 4B). For the transesterification of sunflower oil, the results were more modest. The best result in microwaves compared to conventional heating was observed with reaction time of 90 min with conversion of 24.1% (MW with *tert*-butanol) and 19.4% using the CH.

The results obtained are in agreement with literature. Recent studies have shown the beneficial effect of microwaves on the rates of enzymatic transesterification of vegetable oils. Nogueira and co-workers investigated the effect of microwave irradiation on the rate of transesterification of macauba oil with ethanol catalyzed by Novozyme 435 and Lipozyme IM (*Mucor miehei*).The results showed that the activity is increased about one order of magnitude due to microwave. Were obtained 45.2% with Novozyme (30 °C, 2.5% enzyme loading, 15 min of reaction) and 35.8% with Lipozyme IM (40 °C, 5% enzyme loading, 5 min of reaction).[12] However, the maximum conversions achieved in that study was significantly lower than the 100% conversion. In other study, compared to CH, MW significantly

increased the transesterification rate of soybean oil with methanol catalyzed by Novozyme 435: under the optimum conditions (a_w of 0.53, *tert*-amyl alcohol/oil volume ratio of 1:1, methanol/oil molar ratio of 6:1, 3% Novozym 435 and 40 °C), a yield in FAME of 94% could be achieved in 12 h using MW, compared to 24 h using CH.[29] The microwave-assisted enzymatic synthesis of beef tallow biodiesel also was studied. Under conventional heating, a full conversion of beef tallow into ethyl esters was achieved in 48 h, while the reaction reached equilibrium in 8 h using microwave heating with the same yield (conditions: 45 °C, using beef ethanol:oil molar ratio of 9:1, 20% enzyme loading), indicating that to achieve the same yield of FAEE, a shorter time was needed under microwave irradiation compared to conventional heating.[43]

In terms of transesterification activity, the values found for Novozyme 435 in MI were 695 and 273 U g^{-1} for soybean and sunflower oils, respectively, which represents residual activity (RA) compared to CH of 153 and 125% respectively. Therefore, under MW it was possible an improvement on the reaction rate of approximately 1.5 fold in 90 min, when compared to that

realized under CH using soybean oil. In the order hand, the enzyme showed a decrease in activity with both oils in the FP mode of operation without *tert*-butanol (RA: 39 and 41%) (Table 1).

Table 1. Microwave irradiation (MW) vs. conventional heating (CH) for enzymatic FAEE production. FAEE conversion and activity

	Soybean oil			
	FP*,a	**FP**,a**	**CH*,a**	**PC*,b**
FAEE (%)	15.51+0,80	61.05+1.41	39.87+2.76	9.08+0.45
Activity (U.g^{-1})	176.49	694.83	453.63	230.55
RA (%)	38.91	**153.17**	100.00	50.82
	Sunflower oil			
	FP*,a	**FP**,a**	**CH*,a**	**PC*,b**
FAEE (%)	8.01+0.13	24.13+1.41	19.36+1.45	4.34+0.71
Activity (U.g^{-1})	90.63	273.39	219.33	109.74
RA (%)	41.32	**124.65**	100.00	50.03

*With *tert*-butanol; **Absence *tert*-butanol. [a]90 min; [b]40 min. MI: 50 W. FP: fixed power mode; PC: power cycling mode.

The effect of the microwave absorbing character of the substrate might contribute to the faster reaction rate. It was presumed that microwave heating involved directed energy absorption by the functional groups that bear ionic conductivity or a dipole rotational effect. In the reaction mixture, ethanol may be a good microwave radiation absorption material. Its dipole may quickly reorient under microwave radiation, which would destroy the two-tier structure of the interface between the ethanol and the oil, making the functional groups too reactive. Microwave irradiation might also increase the emulsification speed, which results in an accelerated transport of reactants or products due to the better contact between the enzyme and the substrate, and thus an improved reaction rate.[43] In the order hand, the results show that the tests performed on MW in the absence of co-solvent (only ethanol) indicated a remarked decrease in enzyme activity compared to conventional heating (Table 1, Figure 4). The consistency of these results can be verified by the well-known fact that during the dielectric heating, radiation penetrates the material so that heat transfer takes place from the heart material to the surface. This type of transfer causes heating of the bulk material and a rapid increase in its temperature.[44] A striking feature of the dielectric heating is its selectivity for certain types of materials, especially with polar characteristics and high dielectric constant (parameter that denotes the capability to store electromagnetic energy). One should take into account that ethanol addition to being hydrophilic (Log P = - 0.24) has high dielectric constant (ε' = 24.3). The particular case of mixtures, the preferential heating of certain components can result in the formation of hot spots within the sample, or that is, regions where the temperature is well above the temperature sample mean.[44] Thus, the use of ethanol can lead to the creation of micro-hot spots in the system leading to denaturation of the enzyme.

In this sense, the *tert*-butanol that is moderate polar solvent (log P = 0.80) and has lower dielectric constant than ethanol (ε' = 12.47), and improve the solubility of the system oil, FAEE, ethanol and glycerol as mentioned in item 3.1, acts as a "protective agent of the enzyme", preventing the formation of micro-hot spots and denaturation of the enzyme by overheating, leaving for the enzyme the beneficial effects of microwaves on their structure. It is also possible that the enzyme behaves slightly differently and becomes more active if a conformational change in the enzyme facilitates the substrate to approach the active site of the enzyme more easily under microwave irradiation than that under conventional heating.

Influence of operation mode microwave reactor on the transesterification activity

Most studies in the literature do not explore the mode of operation of the microwave reactor in enzymatic reactions.[44,12,30] In this sense, the versatility of the microwave reactor was explored by varying the mode of operation of the microwave irradiation (input power 50 W). In addition to the fixed power mode, in which the sample was continuously irradiated, operation modes PC (power cycling) and D (dynamic) were also evaluated.

In the PC mode the system was irradiated for 29, 44 and 88 cycles of 25 seconds each and cooling interval of 45, 30 and 15 seconds, respectively, resulting in 34; 40 and 59 min of total reaction times, respectively. For all cycles, the cooling time was about 22 min and cycle times of irradiation were 12, 18 and 37 min. It is noteworthy that during the irradiation cycle the cooling system is down which can lead to formation of "hot spots" in the reaction, this fact justifies the tendency to decrease in the enzymatic rate of formation of FAEE with number of cycles greater than 44 (40 min) with both oils (Figure 5). In the PC mode the residual activity was 50% for transesterification of both oils (Table 1).

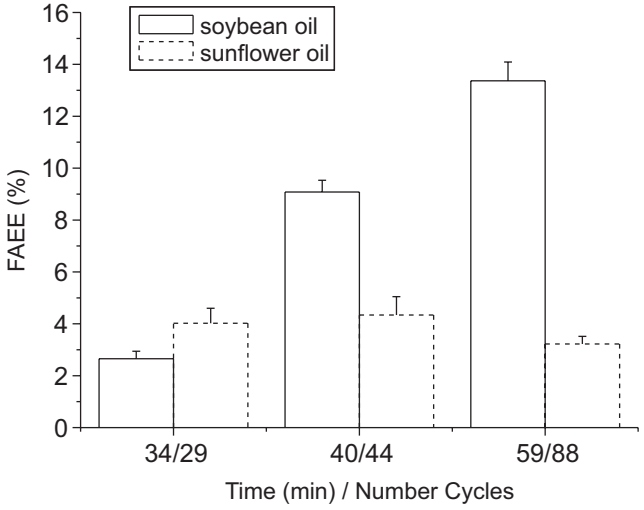

Figure 5. *FAEE production by enzymatic transesterification of vegetable oils under MW by power cycling mode (PC) with tert-butanol. Conditions: 60 °C, 3.36% enzyme loading*

In the dynamic method a single stage of irradiation has been defined. The sample is irradiated only in the initial steps of the reaction to reach the set temperature. In this operation mode, the sample was irradiated at 50 W of power to the temperature set (60 °C), which was reached after about 42 seconds of irradiation. The total reaction time was 22 min. The transesterification of soybean oil and sunflower in this mode of operation provides conversion of 5.42% and 6.69%, respectively. In this operation mode it was not observed activation of the enzyme, probably due to overloading of irradiation in the initial instants of the reaction leading to denaturation of the enzyme.

CONCLUSION

Novozyme 435 was successfully activated for transesterification of vegetable oils via microwave. It was observed a significant effect of the association of *tert*-butanol and microwave irradiation on the enzymatic catalytic activity. The results showed that the use of microwave operating at fixed power mode improves the enzymatic activity (RA = 153%, with soybean oil). Soybean oil was 100% converted to FAEE in just 3 h of reaction using microwave heating (fixed power mode), while under conventional heating the conversion was about 57% at similar reaction conditions. Microwave presents itself as an important ally to the conventional enzymatic catalysis in order to make practical application of lipases in industrial scale for biodiesel production.

ACKNOWLEDGEMENTS

The Authors are grateful for the financial support from the Brazilian research funding agencies CAPES, CNPq and FAPITEC/SE.

REFERENCES

1. Treichel, H.; Oliveira, D.; Mazutti, M. A.; Luccio, M. D.; Oliveira, J. V.; *Food Bioprocess Technol.* **2010**, *3*, 182.
2. Aarthy, M.; Saravanan, P.; Gowthaman, M. K; Rose, C.; Kamini, N. R. *Chem. Eng. Res. Des.* **2014**, *92*, 1591.
3. Clark, J. H.; Budarin, V.; Deswarte, F. E. I.; Hardy, J. J. E.; Kerton, F. M.; Hunt, A. J.; Luque, R.; Macquarrie, D. J.; Milkowski, K.; Rodriguez, A.; Samuel, O.; Tavener, S. J.; White, R. J.; Wilson, A. J.; *Green Chem*. **2006**, *8*, 853.
4. Helwani, Z.; Othman, M. R.; Aziz, N.; Fernando, W. J. N.; Kim, J.; *Fuel Process. Technol.* **2009**, *90*, 1502.
5. Leung, D. Y. C.; Wu, X.; Leung, M. K. H.; *Appl. Energy* **2010**, *87*, 1083.
6. Antczak, M. S.; Kubiak, A.; Antczak, T.; Bielecki, S.; *Renewable Energy* **2009**, *34,* 1185.
7. Fjerbaek, L.; Christensen, K. V.; Norddahl, B.; *Biotechnol. Bioeng.* **2009**, *102*, 1298.
8. Tan, T.; Lu, J.; Nie, K.; Deng, L.; Wang, F.; *Biotechnol. Adv.* **2010**, *28*, 628.
9. Kumari, A.; Mahapatra, P.; Garlapati, V. K.; Banerjee, R.; *Biotechnol. Biofuels* **2009**, *2*, 1.
10. Robles-Medina, A.; Gonzalez-Moreno, P. A.; Esteban-Cerdan, L.; Molina-Grima, E.; *Biotechnol. Adv.* **2009**, *27*, 398.
11. Vieitez, I.; Silva, C.; Alckmin, I.; Borges, G. R.; Corazza, F. C.; Oliveira, J. V.; Grompone, M. A.; Jachmanián, I.; *Renewable Energy* **2010**, *35*, 1976.
12. Nogueira, B. M.; Carretoni, C.; Cruz, R.; Freitas, S.; Melo Jr., P. A.; Costa-Félix, R.; Pinto, J. C.; Nele, M.; *J. Mol. Catal. B: Enzym.* **2010**, *67*, 117.
13. Halim, S. F. A.; Kamaruddin, A. H.; *Process Biochem.* **2008**, *43*, 1436.
14. Ognjanovic, N.; Bezbradica, D.; Knezevic-Jugovic, Z.; *Bioresour. Technol.* **2009**, *100*, 5146.
15. Hayes, H. B. L.; *Microwave Synthesis: Chemistry at Speed of Light*, CEM Publishing: Mathews, 2003.
16. Kappe, C. O.; *Microwave in Organic and Medicinal Chemistry*, Wiley-VCH: Weinheim, 2005.
17. Fortuny, M.; Oliveira, C. B. Z.; Melo, R. L. F. V.; Nele, M.; Coutinho, R. C. C.; Santos, A. F.; *Energy Fuels* **2007**, *21*, 1358.
18. Fortuny, M.; Ramos, A. L. D.; Dariva, C.; Egues, S. M. S.; Santos, A.F.; Nele, M.; Coutinho, R. C. C.; *Quim. Nova* **2008**, *31*, 1553.
19. Herrero, M. A.; Kremsner, J. M.; Kappe, C. O.; *J. Org. Chem.* **2008**, *73*, 36.
20. Bachu, P.; Gibson, J. S.; Sperry, J.; Brimble, M. A.; *Tetrahedron: Asymmetry* **2007***, 18,* 1618.
21. Parvulescu, A. N.; Der Eycken, E. V.; Jacobs, P. A.; De Vos, D. E.; *J. Catal.* **2008**, *255*, 206.
22. Lin, G.; Lin, W.; *Tetrahedron Lett.* **1998**, *39*, 4333.
23. Bradoo, S.; Rathi, P.; Saxena, R. K.; Gupta, R.; *J. Biochem. Biophys. Methods* **2002**, *51*, 115.
24. Matos, L. M. C.; Leal, I. C. R.; de Souza, R. O. M. A.; *J. Mol. Catal. B: Enzym.* **2001***, 72*, 36.
25. Horchani, H.; Chaâbouni, M.; Gargouri, Y.; Sayari, A.; *Carbohydr. Polym.* **2010**, *79*, 466.
26. Sajgure, A.; Yadav, G.; *New Biotechnol.* **2009**, *25S*, S141.
27. Yadav, G. D.; Lathi, P. S.; *J. Mol. Catal. A: Chem.* **2004***, 223*, 51.
28. Yu, D.; Wang, Z.; Chen, P.; Jin, L.; Cheng, Y.; Zhou, J.; Cao, S.; *J. Mol. Catal. B: Enzym.* **2007**, *48*, 51.
29. Carrillo-Munoz, J-R; Bouvet, D.; Guibé-Jampel, E.; Loupy, A.; Petit, A.; *J. Org. Chem.* **1996**, *61*, 7746.
30. Yu, D.; Tian, L.; Ma, D.; Wu, H.; Wang, Z.; Wang, L.; Fang, X.; *Green Chem.* **2010**, *12*, 844.
31. Ndiaye, P. M.; Franceschi, E.; Oliveira, D.; Dariva, C.; Tavares, F. W.; Oliveira, J. V.; *J. Supercrit. Fluids* **2006***, 37*, 29.
32. Royon, D.; Daz, M.; Ellenrieder, G.; Locatelli, S.; *Bioresour. Technol.* **2007**, *98*, 648.
33. Kaieda, M.; Samukawa, T.; Kondo, A.; Fukuda, A. H.; *J. Biosci. Bioeng.* **2001**, *91*, 12.
34. Tamalampudi, S.; Talukder, M. R.; Hama, S.; Numata, T.; Kondo, A.; Fukuda, H.; *Biochem. Eng. J.* **2008**, *39*, 185.
35. Salis, A.; Pinna, M.; Monduzzi, M.; Solinas, V.; *J. Biotechnol.* **2005***, 119*, 291.
36. Kamini, N. R.; Iefuji, H.; *Process Biochem.* **2001**, *37*, 405.
37. Pizarro, A. V. L.; Park, E. Y.; *Process Biochem.* **2003***, 38*, 1077.
38. Soumanou, M. M.; Bornscheuer, U. T.; *Enzyme Microb. Technol.* **2003**, *33*, 97.
39. Li, L.; Du, W.; Liu, D.; Wang, L.; Li, Z.; *J. Mol. Catal. B: Enzym.* **2006**, *43*, 58.
40. Noureddini, H.; Gao, X.; Philkana, R. S.; *Bioresour. Technol.* **2005**, *96*, 769.
41. Perreux, L.; Loupy, A. In *Microwaves in organic synthesis;* Loupy, A, ed.; *Wiley: Weinheim* 2006, p. 134.
42. Loupy, A.; Perreux, L.; Liagre, M.; Burle, K.; Moneuse, M.; *Pure Appl. Chem.* **2001***, 73*, 161.
43. Da Rós, P. C. M.; de Castro, H. F.; Carvalho, A. K. F.; Soares, C. M. F.; Moraes, F. F.; Zanin, G. M,; *J. Ind. Microbiol. Biotechnol.* **2012**, *39*, 529.
44. Berlan, J.; *Radiat. Phys. Chem.* **1995**, *45*, 581.