Development and economic evaluation of an eco-friendly biocatalytic synthesis of emollient esters

Journal:	*Bioprocess and Biosystems Engineering*
Manuscript ID	Draft
Type of Manuscript:	Research Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Serrano-Arnaldos, Mar; University of Murcia, Chemical Engineering Montiel, M. Claudia; University of Murcia, Chemical Engineering Ortega Requena, Salvadora; University of Murcia, Chemical Engineering Máximo, Fuensanta; University of Murcia, Chemical Engineering Bastida, Josefa; University of Murcia, Chemical Engineering
Keywords:	Candida antarctica, Thermomyces lanuginosus, solvent-free, spermaceti, cost assessment

Development and economic evaluation of an eco-friendly biocatalytic synthesis of emollient esters.

Authors: Mar SERRANO-ARNALDOS*, María Claudia MONTIEL, Salvadora ORTEGA-REQUENA, Fuensanta MÁXIMO, Josefa BASTIDA.

Department of Chemical Engineering, University of Murcia, Campus de Espinardo, 30071 Murcia, Spain.

*Corresponding author: mar.serrano@um.es, +34 868887924, https://orcid.org/0000-0001-5919-0951

Acknowledgements: This work has been funded by the Spanish Ministry of Science, Innovation and Universities (CTQ2015-66723-R) and the European Commission (FEDER/ERDF). M. Serrano-Arnaldos and S. Ortega-Requena were beneficiaries of a FPI pre-doctoral scholarship from the Spanish Ministry of Economy and Competitiveness (MINECO) and a Torres Quevedo grant, respectively. We wish to acknowledge D. Ramiro Martínez Gutiérrez (Novozymes Spain S.A.) who kindly supplied the biocatalysts.

Abstract

During the last decades the understanding and prospects of enzyme-catalysed reactions have been massively widened and there are a number of implemented large-scale enzymatic processes mainly based in the use of commercial biocatalysts. As it might happen that the same process can be successfully carried out by different commercial lipases, the election of the biocatalyst must rely on productivity and economic considerations. This work presents productiveness and direct operation cost evaluation as a key tool for the selection between two commercial lipase catalysts, the versatile but expensive Novozym® 435 and a much more economical option, Lipozyme® TL IM, in the synthesis of spermaceti, a mixture of emollient esters with cosmetic applications. Proving that Novozym® 435 leads to minimum savings of 10% with respect to the cheapest immobilized derivative, biocatalyst cost does not appear to be the major contribution to the economics of the processes under study, due to their great capacity to be recovered and reused. At laboratory scale, the biggest economic investment is caused by substrates, which can be massively reduced at industrial scale by using bulk reagents. In such case, energy cost may be the major contribution to the process economy. This work proposes an optimized process ready to be scaled-up in order to accurately determine the energetic requirements of the possible industrial enzymatic synthesis.

Key words: *Candida antarctica; Thermomyces lanuginosus;* solvent-free; spermaceti; cost assessment.

1. Introduction

While the aim of basic scientific research is to contribute to expand the limits of knowledge by setting or revising fundamental principles, the main goal of applied research is to put into practice this elemental basis for the benefit of society. Nevertheless, looking over the wide number of scientific publications existing, it can be noticed that there are little systematic studies dealing with the feasibility of the production of new commodities, either establishing or substantially improving the industrial processes to obtain them. A clear paradigm of that is the assessment of the large-scale use of enzymatic catalysts, even if the great advantages of the enzymatic routes against chemical pathways are well known: lower energy consumption, increased product quality and/or cleaner and eco-friendly processes. In particular, the use of immobilized lipases for obtaining esters with application in food and cosmetic industries has been the subject of a large number of studies since the last years of 20th century [1–3]. However, lipases have not been exploited in its fullness at industrial scale yet, with the exception of a few enterprises, such as Evonik Industries, which commercializes five emollient esters, under the label "enzymatic", produced by using the well-known *Candida antarctica* lipase B derivative Novozym® 435 [4, 5].

Despite lipases are considered one of the most valuable enzymes for synthetic purposes [6], the lack of a widespread biocatalysis-based industry might be chiefly due to the high price of enzymes, that is usually recoup through the immobilization of the lipase and its reuse in several batches. In fact, the investment in an immobilized derivative can be up to 90-95% of the process' cost [7] and so, a large number of viable uses of a biocatalyst are essential to turn the enzymatic synthesis economically profitable in comparison with conventional processes [8]. In this context, one of the objectives of recent research is the development of more active and stable enzymatic catalysts in order to maximize their reuse while reducing their cost [9]. Nonetheless, in view of the number of biocatalysts currently commercialized, it seems that these efforts are still ongoing.

One of the main producers of enzymatic derivatives, Novozymes [10], offers immobilized lipases from different origins adsorbed onto polymeric carriers. Novozym® 435 stands out among them owing to its high performance both in hydrolysis and synthesis reactions under solvent-free conditions or in organic solvent media [11–13], but also because of its expensive market price. On the other hand, Lipozyme® TL IM, which contains a fungal lipase from *Thermomyces lanuginosus*, may be less active but has a much lower cost [9].

Since the increasing number of publications proving the feasibility of the lipase-based esterification of fatty acids with cetyl alcohol gives an idea of the industrial importance of such wax esters [14–17], which are mainly used in cosmetic and pharmaceutical industries due to their emollient properties. As traces of organic solvents in the final product are highly restricted when used for such purposes, the solvent-free synthesis has been proposed to overcome this limitation [18].

In view of the above mentioned, this paper deals with the one-step solvent-free biosynthesis of the four cetyl esters of the spermaceti, a waxy substance formerly obtained from the head cavity of the sperm whale (*Physeter macrocephalus*). Spermaceti is composed of a mixture of cetyl esters and has been synthesised by following its natural proportions [19]. The enzymatic synthesis of such esters is a well-known process and can be developed by using lipases from different origin with high yields [20]. Consequently, the enzymatic production this specialty chemical is evaluated from a technical and an economic point of view in order to establish the possibility of implementing the process at industrial scale. For that purpose, some of the main factors contributing to the manufacturing expenses have been pondered, such as reactor's operation needs, biocatalyst cost and raw materials purchase. The reusability of the two enzymatic catalysts in successive synthesis batches has been taken into consideration [21].

2. Materials and methods

2.1 *Materials*

Novozym® 435 is made of the isoenzyme B from *Candida antarctica* (CalB) immobilized by adsorption onto the macroporous DVB/methacrylate co-polymer resin Lewatit® VP OC 1600. Its declared activity is 10000 PLU/g, where one unit corresponds to the synthesis of 1 µmol per minute propyl laureate from lauric acid and 1-propanol. Lipozyme® TL IM is an immobilized derivative of the fungal lipase *Thermomyces lanuginosus* (Tll) on a cationic silicate (250 UIN/g, interesterification units). Both were kindly donated by Novozymes Spain S.A.

Lauric acid (99%) was purchased at Acros Organics (Geel, Belgium); myristic acid (98%), palmitic acid (98%), stearic acid (95%) and cetyl alcohol at Sigma-Aldrich (Missouri, USA). The rest of chemicals used were all analytical reagent grade.

2.2 Methods

2.2.1 Synthesis under vacuum

The solvent-free synthesis of the spermaceti analogue was optimized in a Parr 5100 series tank reactor (Parr Instrument Co., Illinois, USA), which is provided with a 100 mL vessel heated with a circulating water jacket and a turbine-type vertical stirrer, set at 350 rpm by the reactor's controller. All the assays were performed under an inert atmosphere composed of a 54 L/h flow of N_2 from the autonomous generator 3848 NitroFlow Lab (Parker, Ohio, USA) and low pressure (21.3 kPa) conditions, which were optimized by the authors in previous work [22]. 20 g of substrates were used in all cases. Biocatalyst's concentrations (w/w, referred to substrates) ranging from 0.63% to 5% for Novozym® 435, or 10% for

Lipozyme® TL IM, were tested during the synthesis of the cetyl esters separately (1:1 molar ratio, 70 ºC). The influence of the temperature was studied in the one-step production of spermaceti, by using the best enzymatic catalyst concentration at temperatures comprised between 60 to 80 ºC. Reaction mixture was composed of the substrates' amounts needed to obtain a final product with a 15.3% of cetyl laurate, 37.7% of cetyl myristate, 38.8% of cetyl palmitate and 8.2% of cetyl stearate [19]. The evaluation of the biocatalysts reuse was performed for the synthesis of spermaceti under best reaction conditions. After one hour of use, the immobilized derivative was recovered and rinsed with 3 x 10 mL of acetone, filtering and air drying before using them in another batch, with new fresh reagents.

2.2.2 Acid value determination

The reaction course can be followed by measuring the acid value, A.V., of samples withdrawn from reaction media, which is defined as the amount of KOH, in milligrams, necessary to neutralize the free carboxyl groups present in 1 g of sample [23].

Conversion can be stated as:

$$Conversion(\%) = \frac{A.V._0 - A.V._t}{A.V._0} \times 100$$

Where $A.V._0$ and $A.V._t$ are the acid values at reaction's beginning and at a specific sampling time, respectively. Results are the mean of two data and they are reported with their standard deviation.

3. Results and discussion

3.1 Process technical optimization

3.1.1 Influence of biocatalyst concentration

As described in literature, the high cost of enzymes may be one of the limiting factors for the industrial application of biocatalytic systems [7, 8, 21]. This can be reduced by using the right quantity of an immobilized enzyme, which might compromise both technical (adequate space-time yield) and economic requirements.

Figures 1 and 2 represent the esterification process of cetyl alcohol with lauric, myristic, palmitic and stearic acid catalysed by different concentrations of Novozym® 435 and Lipozyme® TL IM. If both biocatalysts are studied independently, it can be observed that conversion evolves with time mostly in a nonlinear way and results are quite similar independently of the ester synthesized for each enzymatic product. This is in accordance with previous studies, which has proven that even if lower the reaction rates can be noticed

as the substrates' chain length increases, such differences were so little for a given amount of immobilized lipase that, for practical purposes, they do not lead to the preferential synthesis of some esters against the others and do not compromise their simultaneous production [20].

However, significant differences in reaction rate are evident if comparing both biocatalysts under same reaction conditions, being quite lower for the Tll derivative. This must be mainly caused by the hydrophilic character of Lipozyme® TL IM's carrier, which is not so suitable for such hydrophobic reagents and products. Similar findings had been described in the literature, during the synthesis of flavouring esters in organic solvents [24] or oleic acid and methyl ricinoleate estolides [25], but in those cases, the results were explained by the 1,3- positional selectivity of Tll. On the contrary, Novozym® 435 displays a great synthetic activity, due to the hydrophobic nature of its acrylic matrix as well as to the origin of the lipase, since it has been reported that CalB is very active with a wide variety of esters [26].

Despite this, similar product yields (~98.5%) are achieved with this two immobilized lipases, if allowed the time needed according to the kinetics of the process. Thus, a concentration of biocatalyst of 2.5% (0.5 g) was selected as the most suitable for Novozym® 435, while a 5% (1 g) was chosen for Lipozyme® TL IM.

(Figure 1)

(Figure 2)

3.1.2 Effect of the temperature

Operation temperature has a key role in the synthesis of fatty acid esters in solvent-free systems, as the reduction of the viscosity of the reaction media cannot be attained through the use of organic solvents, but through the selection of a temperature that reduces mass transfer limitations while safeguarding the enzyme's functional conformation. In this sense, the one-step synthesis of the spermaceti analogue was carried out at 60, 70 and 80 ºC. Lower temperatures were not tested due to the high melting points of the substances implied in the process, while values above 80 ºC were not considered because of energy consumption.

Figure 3 a shows results for Lipozyme® TL IM, where it can be observed a loss of biocatalyst's activity between 70 and 80 ºC, pointing out that this immobilized derivative may be less resistant to temperature. Even if Tll is known as a thermostable lipase [9], these observations are in agreement with findings from other authors, which have reported the adverse consequences of temperature in Lipozyme® TL IM performance during esterification [24], alcoholysis [27] or transesterification [28] processes.

In contrast, the above mentioned positive effect of temperature is reflected in the conversions reached during the simultaneous synthesis of a spermaceti analogue with Novozym® 435 (Figure 3 b), where an improvement of ~11 percentage units in the conversions is obtained at 30 min as temperature increases from 60 to 80 ºC.

Hence, and taking into account that similar final conversions (>97.5%) were obtained independently of the temperature used, the optimum value was set at 70 ºC, as this temperature reduces the time required to the reagents melting before starting the esterification process.

(Figure 3)

3.1.3 Reusability of the commercial biocatalysts

As previously stated, an operational stability that enables the biocatalyst reuse in the greatest number of consecutive batches is of paramount importance for a successful implementation of the enzymatic pathway at industrial scale. Consequently, Lipozyme® TL IM and Novozym® 435 have been tested in the synthesis of espermaceti under best reaction conditions up to 15 times, with the outcomes showed in Table 1. While losses of biocatalyst mass of 9.6% for Lipozyme® TL IM and 2.8% for Novozym® 435 happened between the first and the last batch, this has no noticeable effect in the enzymatic activity during the consecutive uses. Therefore, yields of ~95% can be achieved in only one hour, and both biocatalysts can be successfully reused, at least, in 14 additional cycles.

Table 1 Amount of biocatalyst employed in the first use and recovered along 15 batches and conversions obtained under optimum reaction conditions (5% w/w of Lipozyme® TL IM - 2.5% w/w of Novozym® 435, 70 ºC, 21.3 kPa, 54 L/h N_2, 350 rpm, 20 g of substrates)

Uses	Conversion (%)		Amount of biocatalyst (g)	
	Lipozyme® TL IM	**Novozym® 435**	**Lipozyme® TL IM**	**Novozym® 435**
1st	93.6 ± 0.9	96.3 ± 1.0	1.0004 ± 1.10^{-4}	$0.5001 \pm 1\times10^{-4}$
5th	94.4 ± 2.0	96.0 ± 1.4	0.9867 ± 2.10^{-3}	$0.5081 \pm 9\times10^{-3}$
10th	94.2 ± 1.6	95.5 ± 1.3	0.9991 ± 7.10^{-3}	$0.4919 \pm 3\times10^{-3}$
15th	93.4 ± 1.4	95.0 ± 0.6	0.9044 ± 8.10^{-3}	$0.4863 \pm 6\times10^{-3}$

These results are in agreement with the ones obtained by Dianóczki et al. [29], where Novozym® 435 was successfully reused up to 10 times in the synthesis of conjugated linoleic acid esters under similar conditions without significant loss of the biocatalyst mass

or activity. On the other hand, Martins et al. [24] has reported the loss of activity of both biocatalysts after the first (Lipozyme® TL IM) and the 7th (Novozym® 435) batch when used in a solvent-free process to obtain isobutyl propionate, putting into evidence that long chained reagents helps to preserve the lipase-based catalysts' activity.

3.1.4 Process productivity

Considering that the heterogeneous process under study is carried out under solvent-free conditions, process productivity has been calculated as the amount of product obtained per gram of biocatalyst and hour by using the conversions attained in the first use of the commercial derivatives during the reusability test under best reaction conditions (Table 1). According to this, the use of 1 g (5% w/w) of Lipozyme® TL IM leads to productivities of 18.7 $g_{product}/(g_{biocatalyst} \cdot h)$ while process productivity when using 0.5 g (2.5% w/w) of Novozym® 435 is of 38.5 $g_{product}/(g_{biocatalyst} \cdot h)$, ~2 times higher than Tll derivative. These results are in accordance with the ones observed in Sections 3.1.1 and 3.1.2, making evident again that the characteristics of Novozym® 435 are better to the esterification process of such hydrophobic reagents.

3.2 Biocatalyst election based in cost estimation

In view of the foregoing it can be concluded that CalB biocatalyst is a more adequate candidate for spermaceti production from a technical point of view. Still, Tll derivative shows an acceptable synthetic activity. Considering that the industrial viability of a biocatalytic pathway lies in its economic profitability, a cost assessment of the optimized production of spermaceti with Lipozyme® TL IM and Novozym® 435 has been also performed. With this aim, only direct operation costs have been taken into account, namely the raw materials (substrates and immobilized derivatives) and utilities (heating, nitrogen, vacuum and stirring) needs, as they will be the only ones affected by the biocatalyst selected. Capital investment, fixed and indirect operation costs have not been considered because they are essentially the same in these two processes.

In this respect, Table 2 gathers the prices of the raw materials and their supplier. It must be noted that none of the suppliers of the reagents used sells both of the biocatalysts, so the authors contacted privately Novozymes Spain S.A., which gently provided the guide prices for Lipozyme® TL IM and Novozym® 435 collected in Table 2. Besides, Table 3 shows the power consumption of the equipment employed during the reactions, as described in Materials and Methods: a thermostatic bath, reactor's controller and stirrer, a N_2 generator and a vacuum pump. In order to avoid calculations relying on consumption data furnished by the suppliers of the equipment and hypothetical cost determinations, real-time measurements of the current intensity were carried out by means of a clamp meter. The energy demanded for the start-up of the system and the maintenance of the operating conditions has been considered, assuming that the average tension at the terminals of the

equipments is 220 V. In the case of the thermostatic bath, 10 min are required in continuous operation to reach the temperature set, 70 ºC, a time used to melt and homogenize the reaction mixture. After that, temperature's controller starts operating in an auto on/off mode, and as a result, its power consumption diminishes. Regarding the N_2 generator, the device requires 35 min to produce enough pressure (6701.3 kPa) to guarantee that an appropriate inert atmosphere is supplied for one hour; then, the generator reconnects for 15 min to raise the pressure, allowing, again, to one hour of autonomy. Given that electricity's price at the moment when measurements were made was 0.2086 €/kWh [30], a realistic power cost was estimated for the enzyme-based synthesis at laboratory scale (Table 3).

Table 2 Costs and providers of the substrates and biocatalysts used

Materials	Price (€/kg)	Supplier
Novozym® 435	1300	Novozymes [10]
Lipozyme® TL IM	70	(Gifts, prices communicated personally)
Cetyl alcohol	41.7	Aldrich (H6800) [31]
Lauric acid	34.2	Acros Organics (10398103) [32]
Myristic acid	37.4	Sigma-Aldrich (70082) [33]
Palmitic acid	42	Aldrich (W283207) [34]
Stearic acid	42	Aldrich (W303518) [35]

Table 3 Power consumption and cost of the manufacturing utilities

Equipment	Specifications	Current intensity (A)	Power (W)	Cost (€/min)
Reactor stirrer (Parr A1120HC)	Magnetic drive Stirrer motor 1/8 HP variable speed 4-Blade turbine type impeller, 35 mm	0.3	66	0.2×10^{-3}
Reactor controller (Parr 4875)	Power controller for handling heating, cooling, safety, and motor control devices.			
Vacuum pump (KNF N816.3KN18)	Max. permissible operating pressure: 0.5 bar Delivery rate: 16 L/min (atm. pressure)			
N_2 generator (Parker 3848 NitroFlow Lab)	Max. flow rate: 32 L/min	*Initial generation and maintenance*: 4.5	990	3.4×10^{-3}
Reactor's heated circulating bath (PolyScience)	Max. capacity: 6 L Flow rate: 7-15 L/min Heater wattage: 1000 W	*Initial:* 8.5 *Maintenance:* 2.4	1870 527	6.5×10^{-3} 1.8×10^{-3}

Once obtained the unit costs for raw materials and process equipment, direct operation cost has been weighted for the previously described optimized production of spermaceti with the Tll and CalB commercial derivatives. The following calculation bases have been established for this purpose: (1) 20 g of final product (in accordance with the scale used during the experimentation), (2) the minimum operation time needed to achieve a conversion of 98%. Putting into evidence, once again, the considerable differences in the activity of these biocatalysts, these processing times are quite unequal between them. Thereby, 103 min are required for Lipozyme® TL IM and 51 min for Novozym® 435, although the double amount of biocatalyst is used in the first case (5% and 2.5% w/w referred to substrates, respectively). Results are presented in Table 4, where the following cost items have been compiled:

1. Substrates cost, which is the same for both processes and is based on the percentage composition of natural spermaceti.
2. Biocatalyst cost, taking into account the optimum concentration of each one and their reusability: 15 batches (minimum) without an apparent loss of activity.
3. Power cost related to the thermostatic bath employed for heating the glass-jacketed reactor's vessel during the conditioning of the reagents (10 min) and the process time.
4. Power cost due to the reactor's controller and stirrer, as well as to the vacuum pump, devices that were connected together to the electrical outlet.
5. Power cost resulting from using the N_2 generator. It is noteworthy that after initial generation (35 min), a single extra run is necessary for the reaction catalysed by Lipozyme® TL IM, as it requires more than an hour (103 min) to reach a conversion of 98%. On the other hand, no further launching of this system is needed for completing the synthesis with Novozym® 435.

Even though it is obvious that the direct operation expenses proposed in Table 4 are far distant from the final cost of the whole process, in the last row these processing costs are given as percentage relatives, assigning 100% to the highest value: Lipozyme® TL IM catalysed synthesis. Surprisingly, direct operation costs for Novozym® 435 are a ~10% lower in spite of the fact that this biocatalyst is almost 20 times more expensive than Tll derivative. This might be explained by the great activity of the commercial immobilized CalB, which contributes to the economic profitability of the biosynthesis in two ways: first, lower concentrations of biocatalyst are necessary and, in addition, it entails shorter operation time and energy consumption.

Table 4 Broken down cost of the synthesis of 20 g of spermaceti at laboratory scale

Cost item	Cost (€/20 g spermaceti)	
	Novozym®435	**Lipozyme TL IM**
Substrates: 20 g Cetyl alcohol: 10.11 g Lauric acid: 1.51 g Myristic acid: 3.73 g Palmitic acid: 3.83 g Stearic acid: 0.81 g	 0.42 0.052 0.14 0.16 0.034	 0.42 0.052 0.14 0.16 0.034
Immobilized lipase Reuse (15 times)	(0.5 g) 0.043	(1 g) 0.0047
Reactor heated circulating bath (70 ºC)	Initial (10 min): 0.065 Maintenance (51 min): 0.09	Initial (10 min): 0.065 Maintenance (103 min): 0.18
Reactor controller and stirrer (350 rpm) and vacuum pump (21.3 kPa)	(51 min) 0.01	(103 min) 0.02
N_2 generator (54 L/h)	Initial (35 min): 0.12 Maintenance: 0	Initial (35 min): 0.12 Maintenance (15 min): 0.051
Total direct operation costs	**1.13**	**1.25**
Percent direct operation costs (%)	**(90.4%)**	**(100%)**

Figure 4 represents all those elements grouped by categories, making patent that the contribution of the biocatalyst to the global cost is little: 0.4% for Lipozyme® TL IM and 3.8% for Novozym® 435. These values are clearly below the 5% proposed by Tufvesson et al. [21] as the maximum weight of biocatalyst cost for the enzymatic production of specialty chemicals, such as cosmetic ingredients. Needless to say that these results are a consequence of the good operational stability of these immobilized derivatives, which, as it has been proved experimentally, can be reused 14 times at least. As shown by this figure, direct operation costs are mainly dependent on the substrates cost, which represent more than a half of the process' economic requirements. This is primarily caused by the fact that these reagents have been acquired from companies that typically provides chemical products at small-scale, for analysis or research purposes.

(Figure 4)

If the scale-up of the process is desired, substrate's purchase must inevitably turn to bulk raw materials suppliers, with whom discounts might even be negotiated if these substances are bought in large quantities [36]. Some examples of these products' prices

and the minimum order quantity for large scale production are given in Table 5, where the purveyors have been selected taking into consideration that they are able to provide cosmetic grade reagents. Comparing these prices with the ones furnished in Table 2, it can be noticed that they can be up to 40 times less expensive when purchased at larger amounts. If calculations for 20 g of spermaceti are made using these new data, direct operation costs would be dramatically reduced: 0.35 € and 0.47 € for CalB and Tll derivatives respectively. This supposes a 25% of saving if Novozym® 435 is used instead Lipozyme® TL IM. In other words, for large-scale purposes the differences between the two processes would be enhanced by using bulk substrates, making even clearer that, still being more expensive, it is more profitable to choose a biocatalyst with better technical features (higher activity) if reused.

Table 5 Bulk raw materials for the synthesis of spermaceti at large scale

Materials	Price (€/kg)	Minimum Order	Company
Cetyl alcohol (98%)	0.85-2.54	1000 kg	J.C. Enterprises. Maharashtra, India [37]
Lauric acid (99%)	1.69	100 kg	Guangzhou Kaoking Chemical Co. Ltd. Guangdong, China [38, 39]
Myristic acid (99.9%)	2.34	200 kg	
Palmitic acid (99%)	0.85-8.5	1 kg	Guangzhou Yiming Chemical Materials Co. Ltd. Guangdong, China [40]
Stearic acid (99.98%)	1.27	200 kg	Guangzhou Kaoking Chemical Co., Ltd. Guangdong, China [41]

In Figure 5 it can be observed that the main contribution to the direct operating costs is due to power consumption when bulk raw materials are used, which supposes now a 94% of the total for Lipozyme® TL IM and an 81% for Novozym® 435. Energy cost may be still lessen, as calculations has been made considering real-time energy consumption at laboratory scale, being all the equipment connected to a domestic electrical network with non-industrial market prices. In this sense, electricity charges for a medium size industry can suppose half of the household cost [42], but even in this case, power investment would be the major contribution to direct operation cost.

(Figure 5)

So, with a view to save energy at larger production scale, the percentage of the global power cost caused by each of the equipment used has been calculated (Figure 6). These results make patent that in both processes most of the energy is consumed to heat the circulating water going through the reactor's jacket and keeping operating temperature constant. Unfortunately, this parameter is largely restricted to the substrates' melting

point in a solvent-free system, but, as it has been pointed out in Section 3.1.2., it is still possible to carry out the synthesis of spermaceti at 60 ºC, even if this entails a longer conditioning of reaction mixture and, possibly, only a slight benefit for such short reaction times. In spite of that, it is well known that that the economic inversion in operating utilities is reduced when increasing the size of a production plant [43, 44], and particularly in the case of the plant's heating energy requirements [45]. The scale-up of the system would be necessary for an accurate evaluation of the energy expenses of both biocatalytic processes.

(Figure 6)

4. Conclusions

The production of a mixture of cetyl esters similar to natural spermaceti has been optimized by using the commercial biocatalysts Lipozyme® TL IM and Novozym® 435, During this study it has been put into evidence the great activity of Novozym® 435, which can double Lipozyme® TL IM's process productivity under optimal conditions. Nevertheless, the optimized process with both biocatalysts led to conversions higher than 97.5%, making patent that a preliminary economic assessment is needed in order to choose the best immobilized derivative. In this sense, cost analysis has pointed out that the better technical features of Novozym® 435 can compensate its high market price and entail direct operation costs lower than the 20 times cheaper Lipozyme® TL IM if reused. Although an appropriate evaluation of large scale energy consumptions is necessary, results by using bulk raw material prices lead to think that biocatalyst investment may not constitute the main economic requirement of the enzymatic process.

Acknowledgements: This work has been funded by the Spanish Ministry of Science, Innovation and Universities (CTQ2015-66723-R) and the European Commission (FEDER/ERDF). M. Serrano-Arnaldos and S. Ortega-Requena were beneficiaries of a FPI pre-doctoral scholarship from the Spanish Ministry of Economy and Competitiveness (MINECO) and a Torres Quevedo grant, respectively. We wish to acknowledge D. Ramiro Martínez Gutiérrez (Novozymes Spain S.A.) who kindly supplied the biocatalysts.

Conflict of Interest: The authors declare that they have no conflict of interest.

References

1. Antonopoulou I, Varriale S, Topakas E, et al (2016) Enzymatic synthesis of bioactive compounds with high potential for cosmeceutical application. Appl Microbiol Biotechnol 100:6519–6543. https://doi.org/10.1007/s00253-016-7647-9

2. Ye R, Hayes DG, Burton R, et al (2016) Solvent-Free Lipase-Catalyzed Synthesis of Technical-Grade Sugar Esters and Evaluation of Their Physicochemical and Bioactive Properties. Catalysts 6:78. https://doi.org/10.3390/catal6060078

3. Yvergnaux F (2017) Lipases: particularly effective biocatalysts for cosmetic active ingredients. Ocl-Oilseeds Fats Crops Lipids 24:D408. https://doi.org/10.1051/ocl/2017013

4. Grunwald P (2014) Industrial Biocatalysis. CRC Press, Boca Raton (FL)

5. Hilterhaus L, Liese A, Kettling U, Antranikian G (2016) Applied Biocatalysis: From Fundamental Science to Industrial Applications. John Wiley & Sons

6. Daiha K de G, Angeli R, de Oliveira SD, Almeida RV (2015) Are Lipases Still Important Biocatalysts? A Study of Scientific Publications and Patents for Technological Forecasting. Plos One 10:e0131624. https://doi.org/10.1371/journal.pone.0131624

7. Borole AP, Davison BH (2007) Techno-economic analysis of biocatalytic processes for production of alkene epoxides. Appl Biochem Biotechnol 137–140:437–449. https://doi.org/10.1007/s12010-007-9070-2

8. Mustafa A, Karmali A, Abdelmoez W (2016) Optimisation and economic assessment of lipase-catalysed production of monoesters using *Rhizomucor miehei* lipase in a solvent-free system. J Clean Prod 137:953–964. https://doi.org/10.1016/j.jclepro.2016.07.056

9. Fernández-Lafuente R (2010) Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. J Mol Catal B-Enzym 62:197–212. https://doi.org/10.1016/j.molcatb.2009.11.010

10. Novozymes A/S Biocatalysis for a sustainable pharma future - Novozymes. https://www.novozymes.com/en/advance-your-business/pharma. Accessed 9 Jan 2019

11. Bansode SR, Hardikar MA, Rathod VK (2017) Evaluation of reaction parameters and kinetic modelling for Novozym 435 catalysed synthesis of isoamyl butyrate. J Chem Technol Biotechnol 92:1306–1314. https://doi.org/10.1002/jctb.5125

12. Koutinas M, Yiangou C, Osório NM, et al (2018) Application of commercial and non-commercial immobilized lipases for biocatalytic production of ethyl lactate in

organic solvents. Bioresour Technol 247:496–503. https://doi.org/10.1016/j.biortech.2017.09.130

13. Madarasz J, Nemeth D, Bakos J, et al (2015) Solvent-free enzymatic process for biolubricant production in continuous microfluidic reactor. J Clean Prod 93:140–144. https://doi.org/10.1016/j.jclepro.2015.01.028

14. Cirillo NA, Quirrenbach CG, Corazza ML, Pedersen Voll FA (2018) Enzymatic kinetics of cetyl palmitate synthesis in a solvent-free system. Biochem Eng J 137:116–124. https://doi.org/10.1016/j.bej.2018.05.021

15. Lima LCD, Peres DGC, Mendes AA (2018) Kinetic and thermodynamic studies on the enzymatic synthesis of wax ester catalyzed by lipase immobilized on glutaraldehyde-activated rice husk particles. Bioprocess Biosyst Eng 41:991–1002. https://doi.org/10.1007/s00449-018-1929-9

16. Montiel MC, Serrano M, Máximo MF, et al (2015) Synthesis of cetyl ricinoleate catalyzed by immobilized Lipozyme® CalB lipase in a solvent-free system. Catal Today 255:49–53. https://doi.org/10.1016/j.cattod.2014.09.015

17. Serrano-Arnaldos M, Maximo-Martin MF, Montiel-Morte MC, et al (2016) Solvent-free enzymatic production of high quality cetyl esters. Bioprocess Biosyst Eng 39:641–9. https://doi.org/10.1007/s00449-016-1545-5

18. Veit T (2004) Biocatalysis for the production of cosmetic ingredients. Eng Life Sci 4:508–511. https://doi.org/10.1002/elsc.200402148

19. Wellendorf M (1963) Composition of Spermaceti. Nature 198:1086–1087. https://doi.org/10.1038/1981086b0

20. Serrano-Arnaldos M, Bastida J, Máximo F, et al (2018) One-Step Solvent-Free Production of a Spermaceti Analogue Using Commercial Immobilized Lipases. ChemistrySelect 3:748–752. https://doi.org/10.1002/slct.201702332

21. Tufvesson P, Lima-Ramos J, Nordblad M, Woodley JM (2011) Guidelines and Cost Analysis for Catalyst Production in Biocatalytic Processes. Org Process Res Dev 15:266–274. https://doi.org/10.1021/op1002165

22. Ortega-Requena S, Bódalo-Santoyo A, Bastida-Rodríguez J, et al (2014) Optimized enzymatic synthesis of the food additive polyglycerol polyricinoleate (PGPR) using Novozym® 435 in a solvent free system. Biochem Eng J 84:91–97. https://doi.org/10.1016/j.bej.2014.01.003

23. ASTM D974–02e1 (2002) Standard test method for acid and base number by color-indicator titration. ASTM International, West Conshohocken, PA

24. Martins AB, da Silva AM, Schein MF, et al (2014) Comparison of the performance of commercial immobilized lipases in the synthesis of different flavor

esters. J Mol Catal B-Enzym 105:18–25. https://doi.org/10.1016/j.molcatb.2014.03.021

25. Aguieiras ECG, Veloso CO, Bevilaqua JV, et al (2011) Estolides Synthesis Catalyzed by Immobilized Lipases. Enzyme Res 2011:1–7. https://doi.org/10.4061/2011/432746

26. Anderson EM, Larsson KM, Kirk O (1998) One Biocatalyst–Many Applications: The Use of *Candida Antarctica* B-Lipase in Organic Synthesis. Biocatal Biotransformation 16:181–204. https://doi.org/10.3109/10242429809003198

27. Basri M, Kassim MA, Mohamad R, Ariff AB (2013) Optimization and kinetic study on the synthesis of palm oil ester using Lipozyme TL IM. J Mol Catal B Enzym 85–86:214–219. https://doi.org/10.1016/j.molcatb.2012.09.013

28. Choi N, Kim Y, Lee J-S, et al (2016) Synthesis of Fatty Acid Ethyl Ester from Acid Oil in a Continuous Reactor via an Enzymatic Transesterification. J Am Oil Chem Soc 93:311–318. https://doi.org/10.1007/s11746-016-2786-9

29. Dianóczki C, Recseg K, Kővári K, Poppe L (2007) Convenient enzymatic preparation of conjugated linoleic acid alkyl esters with C6–C22 alcohols. J Mol Catal B Enzym 45:45–49. https://doi.org/10.1016/j.molcatb.2006.11.005

30. Comisión Nacional de los Mercados y la Competencia (2016) Boletín de indicadores eléctricos 2016

31. Cetyl alcohol, Sigma-Aldrich. https://www.sigmaaldrich.com/catalog/search?term=Cetyl+alcohol&interface=Product%20Name&N=0&mode=mode%20matchpartialmax&lang=es&region=ES&focus=productN=0%20220003048%20219853286%20219853112. Accessed 3 Sep 2018

32. Lauric acid, 99%, ACROS Organics - Organic Building Blocks Chemicals. https://www.fishersci.es/shop/products/lauric-acid-99-acros-organics-5/p-3736787. Accessed 3 Sep 2018

33. Myristic acid, Sigma-Aldrich. https://www.sigmaaldrich.com/catalog/search?term=Myristic+acid&interface=Product%20Name&N=0&mode=mode%20matchpartialmax&lang=es&region=ES&focus=productN=0%20220003048%20219853286%20219853112. Accessed 3 Sep 2018

34. Palmitic acid, Sigma-Aldrich. https://www.sigmaaldrich.com/catalog/search?term=Palmitic+acid&interface=Product%20Name&N=0&mode=mode%20matchpartialmax&lang=es&region=ES&focus=productN=0%20220003048%20219853286%20219853112. Accessed 3 Sep 2018

35. Stearic acid, Sigma-Aldrich. https://www.sigmaaldrich.com/catalog/search?term=Stearic+acid&interface=Product%20Name&N=0&mode=mode%20matchpartialmax&lang=es&region=ES&focus=productN=0%20220003048%20219853286%20219853112. Accessed 3 Sep 2018

36. Brennan DJ (2004) Developing a Process Industry Culture. Morgan Printing

37. Cetyl Alcohol Fatty Alcohol C1698 Supplier From India. https://www.alibaba.com/product-detail/Cetyl-Alcohol-Fatty-Alcohol-C1698-Supplier_146547456.html. Accessed 6 Sep 2018

38. High quality cosmetic material Lauric acid 99%, Product Details from Guangzhou Kaoking Chemical Co., Ltd. https://kao.en.alibaba.com/product/60399285976-802337594/high_quality_cosmetic_material_Lauric_acid_99_price.html?spm=a2700.8304367.prewdfa4cf.2.207346f37aIcYF. Accessed 6 Sep 2018

39. Myristic acid. https://wholesaler.alibaba.com/product-detail/Soap-Detergent-Cosmetic-Surfactant-myristic-acid_60703624293.html. Accessed 7 Sep 2018

40. Chemical Raw Material Palmitic Acid For Cosmetics. https://www.alibaba.com/product-detail/chemical-raw-material-palmitic-acid-for_60148565501.html?spm=a2700.7724838.2017115.109.4a105a4l77Oi9. Accessed 6 Sep 2018

41. High quality cosmetic material Stearic acid, Product Details from Guangzhou Kaoking Chemical Co., Ltd. https://kao.en.alibaba.com/product/60439480037-802394266/high_quality_cosemtic_material_Stearic_acid.html?spm=a2700.8304367.prewdfa4cf.2.28c79411Vzc7eM. Accessed 6 Sep 2018

42. Eurostat - Tables, Graphs and Maps Interface (TGM). http://ec.europa.eu/eurostat/tgm/refreshTableAction.do?tab=table&plugin=1&pcode=ten00117&language=en. Accessed 13 Dec 2018

43. Jiménez-González C, Constable DJC (2011) Green Chemistry and Engineering: A Practical Design Approach. John Wiley & Sons

44. Vogel GH (2014) Production cost estimation. Ullmanns Encycl. Ind. Chem.

45. Bermúdez JM, Beneroso D, Rey-Raap N, et al (2015) Energy consumption estimation in the scaling-up of microwave heating processes. Chem Eng Process Process Intensif 95:1–8. https://doi.org/10.1016/j.cep.2015.05.001

Figure captions

Fig. 1 Cetyl laurate (a), myristate (b), palmitate (c) and stearate (d) solvent-free synthesis using different quantities of Lipozyme® TL IM (70 ºC, 21.3 kPa, 54 L/h N_2, 350 rpm, 20 g of substrates, 1:1 molar ratio)

Fig. 2 Cetyl laurate (a), myristate (b), palmitate (c) and stearate (d) solvent-free synthesis using different quantities of Novozym® 435 (70 ºC, 21.3 kPa, 54 L/h N_2, 350 rpm, 20 g of substrates, 1:1 molar ratio)

Fig. 3 Spermaceti solvent-free synthesis at different temperatures using 5% w/w of Lipozyme® TL IM (a) and 2.5% w/w of Novozym® 435 (b) (21.3 kPa, 54 L/h N_2, 350 rpm, 20 g of substrates)

Fig. 4 Contribution of the biocatalyst, substrates and power to the direct operation costs of the synthesis of 20 g of spermaceti under optimal conditions at laboratory scale

Fig. 5 Contribution of the biocatalyst, substrates and power to the direct operation costs of the synthesis of 20 g of spermaceti under optimal conditions when bulk raw material prices are considered

Fig. 6 Contribution of the different equipment to the energy costs while synthesizing 20 g of spermaceti

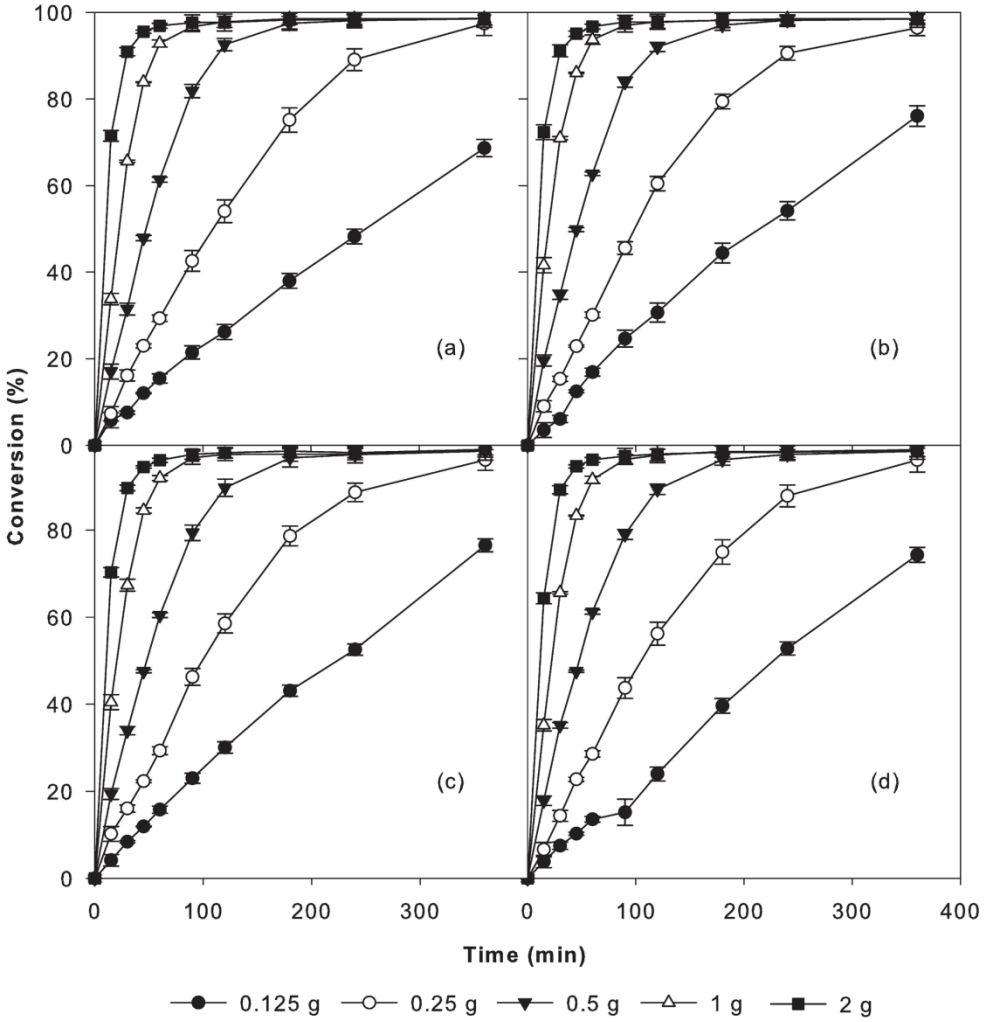

Fig. 1 Cetyl laurate (a), myristate (b), palmitate (c) and stearate (d) solvent-free synthesis using different quantities of Lipozyme® TL IM (70 ºC, 21.3 kPa, 54 L/h N2, 350 rpm, 20 g of substrates, 1:1 molar ratio)

173x181mm (300 x 300 DPI)

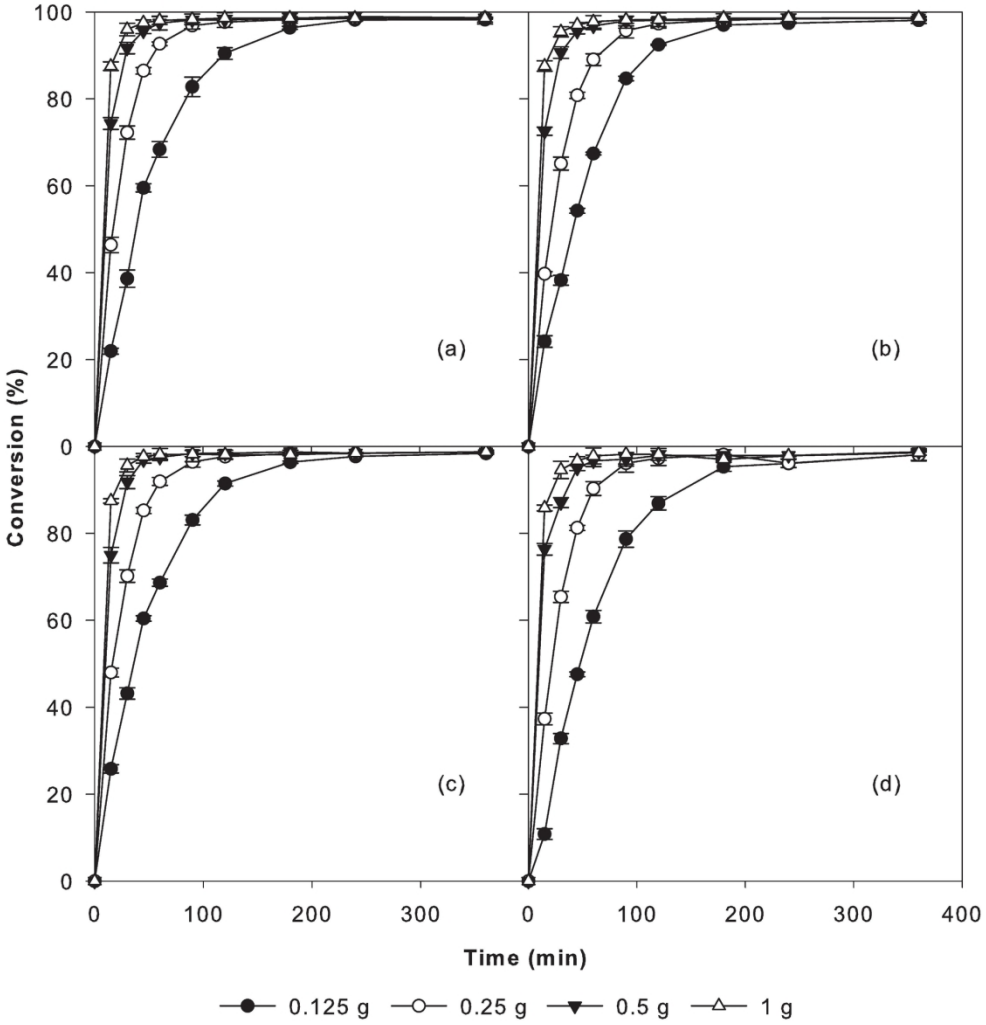

Fig. 2 Cetyl laurate (a), myristate (b), palmitate (c) and stearate (d) solvent-free synthesis using different quantities of Novozym® 435 (70 ºC, 21.3 kPa, 54 L/h N2, 350 rpm, 20 g of substrates, 1:1 molar ratio)

172x181mm (300 x 300 DPI)

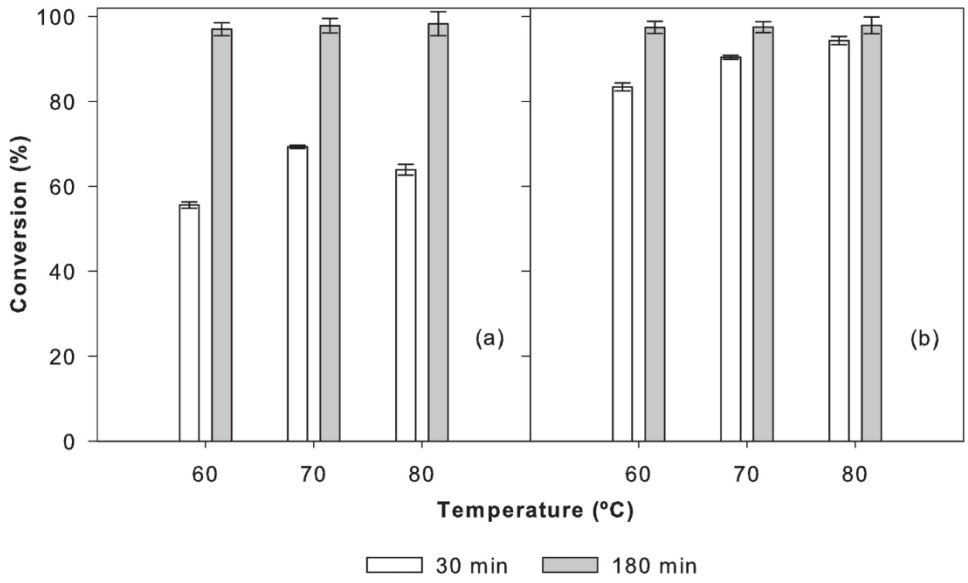

Fig. 3 Spermaceti solvent-free synthesis at different temperatures using 5% w/w of Lipozyme® TL IM (a) and 2.5% w/w of Novozym® 435 (b) (21.3 kPa, 54 L/h N2, 350 rpm, 20 g of substrates)

173x106mm (300 x 300 DPI)

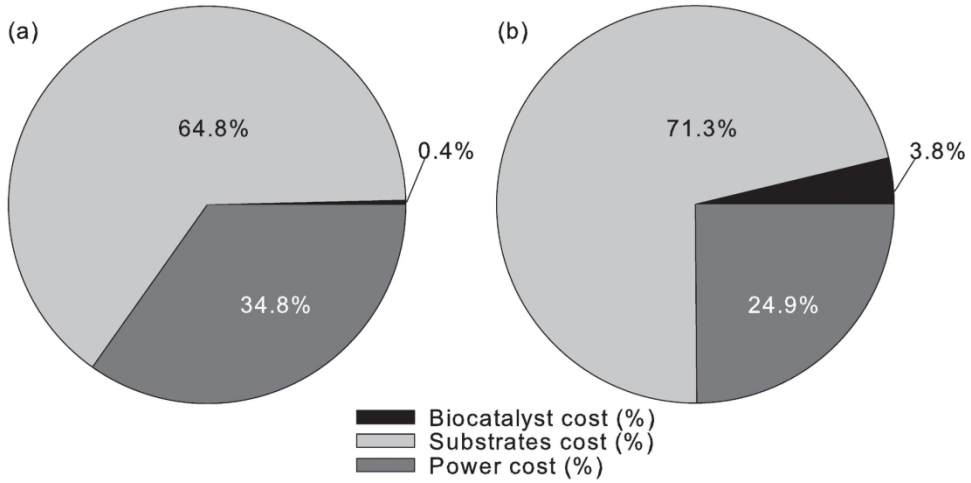

Fig. 4 Contribution of the biocatalyst, substrates and power to the direct operation costs of the synthesis of 20 g of spermaceti under optimal conditions at laboratory scale

163x85mm (300 x 300 DPI)

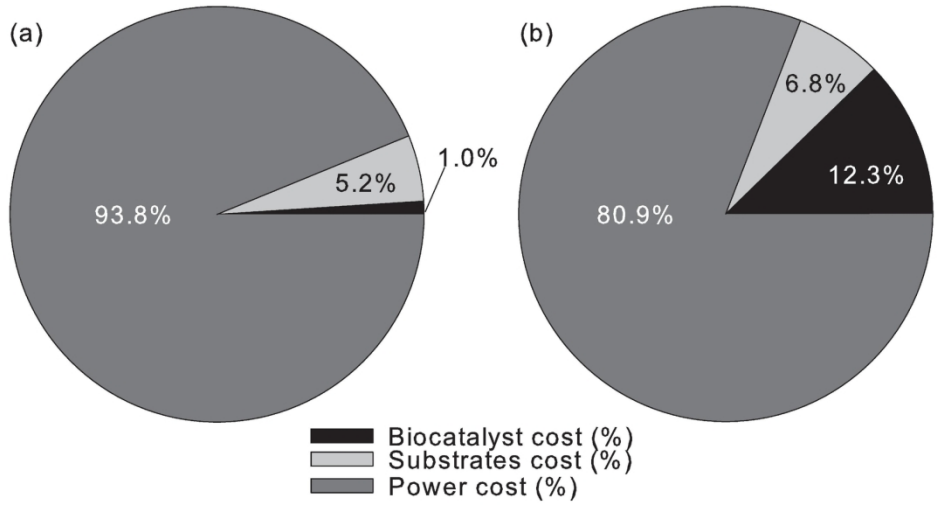

Fig. 5 Contribution of the biocatalyst, substrates and power to the direct operation costs of the synthesis of 20 g of spermaceti under optimal conditions when bulk raw material prices are considered

156x85mm (300 x 300 DPI)

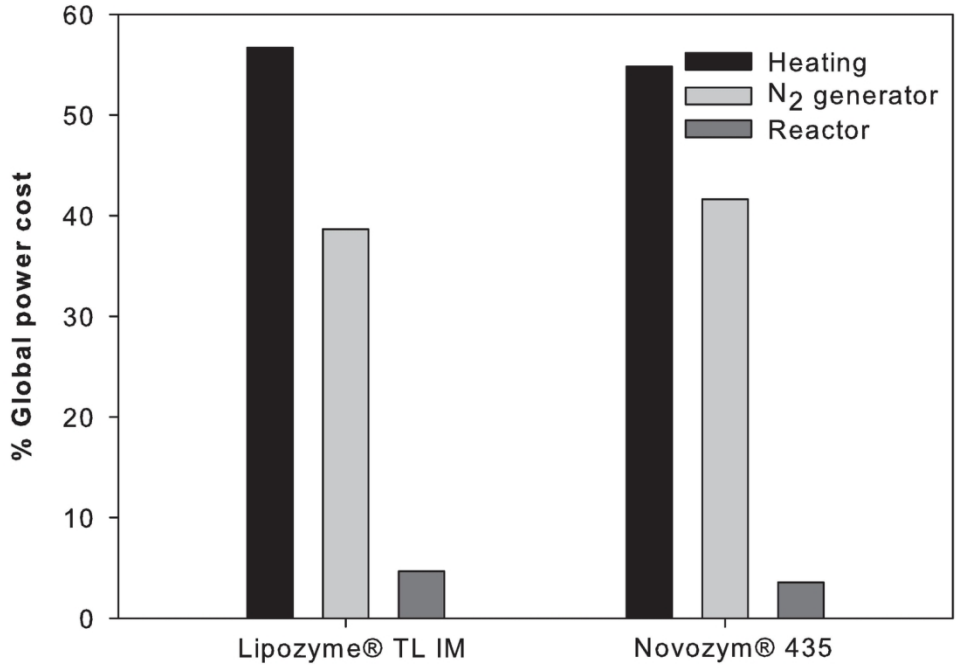

Fig. 6 Contribution of the different equipment to the energy costs while synthesizing 20 g of spermaceti

144x112mm (300 x 300 DPI)