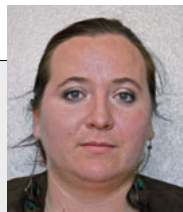Sebastien Elgue   Annelyse Conte

Two-phase enzymatic reaction using Process Intensification technologies

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ABSTRACT

Developing continuous enzymatic process, especially when a two-phase medium is required, remains nowadays a great challenge. So, there is a need to provide a technical solution for generating highly controlled interfacial area. In this study, three types of reactors, conventionally used for chemical process intensification, were tested for continuous lipase-catalysed esterification in two-phase medium. On the single criterion of mass transfer performance that is considered here, the Nitech oscillating reactor proved to be the more efficient to transform the input mechanical power into mass transfer while proposing easily adjustable residence time. Nevertheless Corning and Chart flow reactors were shown to be very suitable candidates even if mass transfer performances are dependant on the flow-rate and therefore are not uncoupled from the residence time. This results in a less convenient choice of operating conditions. Also, it has been shown here that the model reaction could also be a simple way to characterize mass transfer performance of these technologies.

INTRODUCTION

Operation of enzymatic reactions in two-phase medium, *i.e.* oil-water dispersions, is very common when hydrophobic reactants are concerned, as for instance in the case of hydrolysis of lipids (1-2). Moreover, enzymatic reactions, such as esterification, can be effective in two-phase media using free enzymes (3). Indeed, even in a two-phase system with a water activity close to unity, esterification can be effective due to a shift of the thermodynamic equilibrium by maintaining a low ester activity. After preliminary studies, when industrial development is envisaged, continuous operation must be considered in order to increase productivity and economic viability. But, at present time, it is not possible to propose a realistic continuous liquid-liquid reactor where controlled contact area and steady operation is insured, while this would greatly simplify the development of the process. So, usually, to
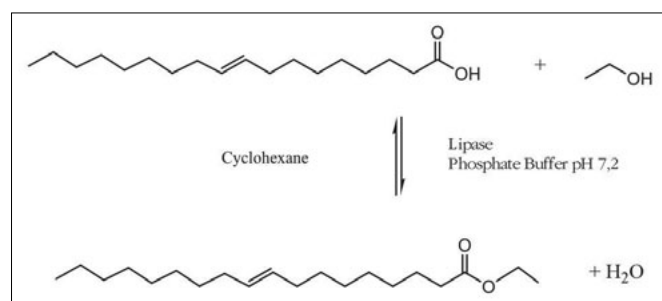
overcome this technical difficulty and make the more efficient and economic use of the biocatalyst, this latter is used as an immobilized biocatalyst packed in a tubular reactor (1, 4, 5). Therefore, there is a need for a technical solution which makes it possible to directly use aqueous enzyme solutions in a continuous reactor, where the two-phase system is well controlled in terms of interfacial area and steadiness of operation. This opens the field to the so-called "intensified" reactors which is now an identified target for successful industrial development of new processes. The aim of this study is to consider the use of several intensified reactor technologies, already existing at commercial scale, to operate enzymatic two-phase reactions. The assessment of the candidates will be done using a model reaction, the conventional lipase-catalysed esterification of oleic acid with ethanol in order to focus on the reactor performances only.

MATERIAL AND METHODS

Description of the reaction

The chosen reaction is a model reaction, the lipase-catalysed esterification with ethanol of a fatty acid, the oleic acid, to yield ethyloleate and water (Figure 1).

To catalyse this esterification, the commercial liquid form of lipase B from *Candida antarctica*, namely Lipozyme CalB L (Novozymes, Bagsvaerd, Denmark), was chosen. Its concentration is 5000 LU/g. To obtain an enzyme activity in agreement with the residence times envisaged in the continuous process, it was necessary to dilute from 50 to 100 times the initial solution in a phosphate buffer 0.1M, pH 7.2. Esterifications are equilibrated reactions, nevertheless the reaction is shifted towards esterification because the hydrophobic ester accumulates in the organic solvent which indeed plays the role of a reservoir for the most hydrophobic reactant, the ester. In this work, cyclohexane has been used to play this role.
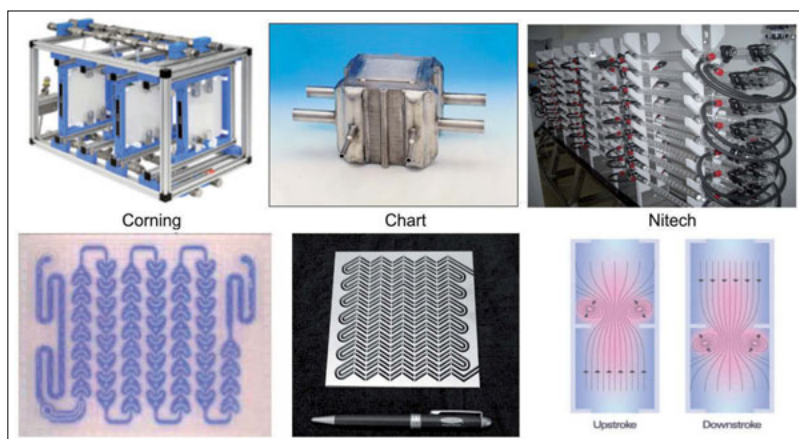

> **Figure 1. Reaction scheme**

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Figure 2. Considered Process Intensification technologies – external and internal designs respectively from left to right for Corning, Chart and Nitech reactors

Material

The use of several flow reactor technologies, already existing at commercial scale, is considered. In fact, for multi-phase applications, flow reactors allow enhanced mass transfer and controlled interfacial area, providing reproducible and reliable operation. From a technical viewpoint, flow reactors are classified into two broad categories:

– Static mixing devices rely on motion of the fluid through the reactor to generate mixing effects and phase dispersion (in two-phase systems). The most common type is the tubular reactor. Heat exchanger reactors also belong to this category.

– Dynamic mixing devices where, by contrast, mixing is carried out using mechanical energy input done by different systems like in the most common type, the continuous stirred tank reactor, where mixing is obtained *via* the use of mechanical stirrers.

Three commercial solutions are considered here (figure 2). The ability to carry out two-phase reactions and the technological maturity have driven the choice of these technologies. Hence, two heat exchanger-reactors, respectively commercialized by Corning (6-9) and Chart Industries (10-11), and the Nitech Solutions COBR™ (12-13) involving dynamic mixing by a pulsation device, were studied. It has been chosen to work on the pilot scale versions of these equipments, in order to be able to easily extrapolate the process to industrial scale. Consequently, the following versions of each reactor have been used:

– Corning AFR Gen 1, with channel width of 4 mm and depth of 0.7 mm.

– Chart Shimtec with channel width of 2mm and depth of 2 mm.

– Nitech COBR with tube diameter of 15 mm and doughnuts diameter of 8 mm.

Methods

The lipase-catalysed esterification of oleic acid with ethanol has been studied at pilot scale, which means that the global flow-rate (*i.e.*, the sum of the flow-rates of both phases) is in the range 15 to 150 g/min. A specific fluidic module has been used for controlling the feeding of raw solutions and the temperature of the process. The fluidic module is constituted of two dosing lines composed of micro annular gear pumps (HNP Mikrosysteme), mass-flowmeters based on the Coriolis effect (Micromotion) and temperature and pressure sensors. PID controllers ensure constant delivering of flow-rates by acting on the motor pump regime. Special care has been taken to prevent from pulsating flow-rates, especially in the case of COBR™.

This is done by using a combination of check and safety valves set on the dosing lines to prevent piston pulsation from disturbing the pump flow-rates. A process-thermostat (Lauda integral XT 150) is associated to the fluidic module to control the process temperature. A sampling valve is located at reactor outlet to estimate the final conversion by HPLC chromatography analysis.

RESULTS AND DISCUSSION

Study of the esterification using batch reactors

In order to have a reference value, batch esterification reactions were performed in small Eppendorf tubes agitated in a Vortex. 90.4 % conversion was obtained within 30 minutes. In addition, to make the comparison as complete as possible, the esterification has also been carried out in a standard glass reactor at lab scale (1L, double jacketed with impeller stirring device) at optimal stirring velocity (360 rpm). In that case, the operating conditions have been chosen to be comparable to those applied in the different continuous reactors. Batch study shows that kinetics (estimated from conversion rate) is 6 times lower in the batch laboratory scale reactor than in small Eppendorf tubes. Such behaviour demonstrates the influence and the limitation of the mass transfer during this reaction in a two-phase medium.

Study of the esterification using static mixing flow reactors

In flow reactors based on the static mixing concept, performances are driven by flow velocity (14-15): an increase of flow-rate favours the generation of interfacial area and therefore mass transfer. Therefore, as reactor volume is fixed, increasing flow-rate reduces the residence time. Consequently, in the case of two-phase reactions, depending of the prominent reaction regime (kinetic, mass transfer or transitional regime), there is an optimal flow-rate leading to the highest conversion level. To validate these assumptions, a study of the global flow-rate influence has been carried out in Corning and Chart flow reactors (figure 3). Other operating conditions are respectively: volumic phase ratio equal to 1, temperature of 30°C, lipase concentration of 100 LU/g and molar ratio between ethanol and oleic acid equal to 3.

The evolution of conversion in respect to global flow-rate in the Corning reactor exhibits a flat bell-shaped curve, with 25% conversion at maximum fixed by the reactor volume (120 mL).
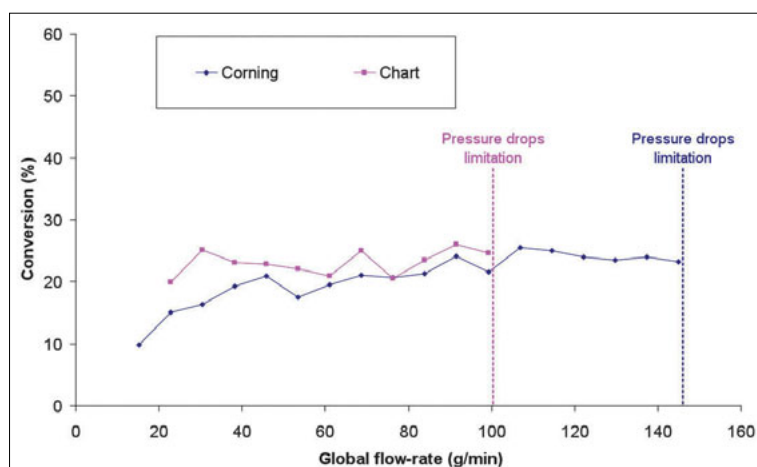

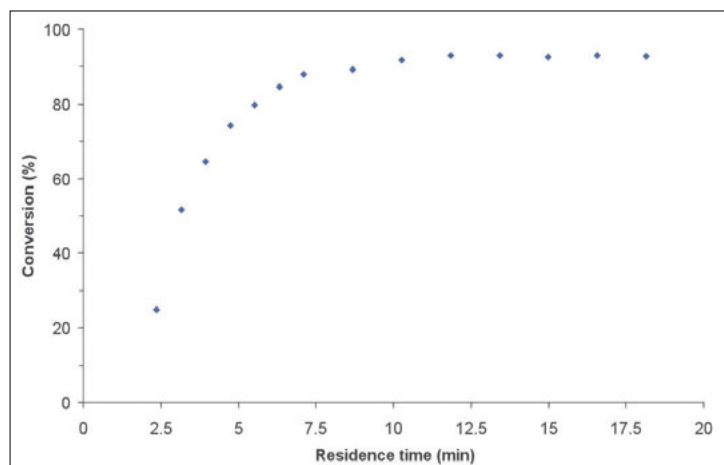Figure 3. Influence of flow-rate on conversion for Corning and Chart flow reactors

Figure 4. Esterification conversion according to residence time in Corning reactor

Figure 5. Evolution of conversion in COBR^{TM} in respect to pulse characteristics

Such a profile complies with the curves reported in literature (14). Nevertheless, in the present case, the profile is sluggish according to the reaction characteristics in terms of mass transfer and of kinetics. At flow-rates lower than 105 g/min, conversion is increasing due to improved mixing performances and interfacial area generation, despite the reduction of global residence time (defined as the reactor volume divided by the global flow-rate). At higher flow rates, the conversion will decrease because reduction of the residence time is more detrimental than the gain in mass transfer which moreover will reach an upper cap. Unfortunately, because of the pressure drops involved and the limitation of dosing line pumps, it was not possible to experimentally observe such behaviour.
For the Chart reactor, the improved mixing and the residence time reduction seem to counterbalance in the range of tested flow rates. This is in agreement with the fact that the Chart reactor offers better mass transfer performances at low flow-rates. As a consequence, the maximal conversion (around 25% according to the reactor volume of 120 mL) was reached for different values of flow-rate. Therefore, to determine the optimal flow-rate, additional criteria should be defined, for instance in terms of productivity or power consumption. Indeed, high flow rates resulted in better productivity but also in high power consumption because pressure drop was larger.
Different parameters have been investigated to compare the associated behaviours of the Corning and Chart reactors. In this way, the influence of temperature, lipase concentration and substrate ratio (molar ratio between ethanol and oleic acid) have been thoroughly studied. These studies emphasize the limitation of the considered lipase catalysed esterification by mass transfer and the possibility of process optimisation.
For these two flow reactors, this parametric study has shown that, in spite of a possible optimisation, the antagonism residence time / mass transfer did not unable high conversion level to be reached in the considered reactor. To estimate the residence time required to obtain high conversion, specific experiments were carried out in both reactors with operating conditions favourable to kinetics: flow-rate of 46 g/min, temperature of 50°C, and substrate ratio of 6. Results (figure 4) emphasize clearly the possibility to reach high conversion levels with moderate increase of residence time, i.e. of reactor volume. Compared to the initial 140 s residence time, this means that such residence times can be easily obtained by using reactors with larger volumes (3 to 4 fold increase). Indeed, such values fully comply with residence time values usually available in Corning or Chart flow reactors. This definitely validates the possibility to operate lipase-catalysed esterification in open-loop intensified flow-reactors. Note that a correlative increase of the pressure drop and therefore of the power consumption must also be taken into account.
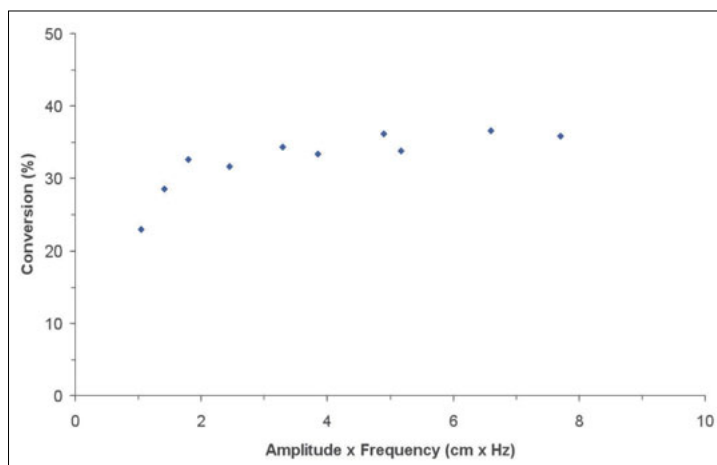
Esterification study in Nitech COBR^{TM}
The equipment is a DN15 version of the reactor, including a piston to provide pulsation. This dimension imposes to increase the global flow-rate compared to the value required in Corning and Chart reactors. However, the length and the volume of the reactor have then been adapted to obtain the same 140 seconds residence time. From its technical principle, the velocity in the Nitech reactor is low (about 0.01 m/s) that entails a poor mixing by the static mixing effect. As a consequence, the mass transfer performances are only governed by the pulsation, through its amplitude and frequency. As it was shown that the reaction occurs in mass transfer regime, the pulsation, characterized by the product amplitude-frequency, has a direct effect on mass transfer and favours the conversion (figure 5).

Conversions as high as 35% are obtained, significantly better than for flow-reactors for the same residence time. In fact, this reactor, thanks to the pulsation system, improves greatly the mass transfer (mainly through greater interfacial area generation) in a range that could not be reached in static mixing based reactors, where a compromise between performance and residence time has to be considered. It is important to note that increasing residence time in the COBR™ is easy to perform. For instance, with a residence time of 350 seconds, conversion was found to be about 60%. This offers interesting perspective when coupled with optimisation of the reaction conditions: temperature, concentration, ethanol equivalent, volumic phase ratio, etc.

Figure 6. Benchmark of mass transfer performances

Benchmarking

The results obtained on different equipments and various operating conditions lead to the same conclusions: the considered lipase catalysed esterification is limited by mass transfer. Therefore, the operation of this model reaction using these different technologies can also be considered as an assessment of the mass transfer performances of each reactor, especially in terms of generation of interfacial area. As the products and the reaction involved here are easy to carry out, this constitutes an excellent test to characterise mass transfer performances of different devices. Indeed, a first approach of benchmarking of equipments could be performed in this way. This was done for the different equipments considered in the present work. A given 140 second global residence time was applied, with temperature of 30°C, lipase concentration of 50 LU/g and molar ratio between ethanol and oleic acid of 3. For COBR™, optimal amplitude and frequency have been chosen as being those leading to the maximal conversion. Figure 6 illustrates the resulting comparison of mass transfer performances of the different equipments. Based on this criterion, Nitech COBR™ appears the most efficient. Moreover, in such a device the possibility to reach high residence time (up to 100 minutes) also goes along with its enhanced mass transfer performances.
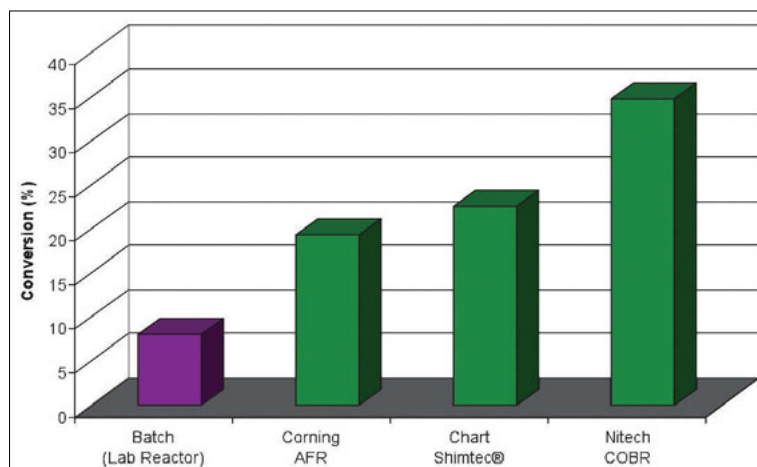
CONCLUSION

The main result of this work was to demonstrate that novel technologies recently proposed for intensification of chemical processes are indeed very suitable for two-phase reactions. This was tested on a model enzymatic reaction but representing an important class of enzymatic reactions where lipases are used for organic synthesis. Three technologies of intensified reactors were tested. From the criterion of mass transfer performances, the Nitech oscillating reactor proved to be the more efficient to transform the input mechanical power into mass transfer performance while proposing easily adjustable residence time. Nevertheless Corning and Chart reactors were shown to be very suitable candidates even if mass transfer performances depend on the flow-rate of the reacting mixture and therefore are not uncoupled from the residence time. This results in a less convenient choice of operating conditions. But such technologies are likely to be more reliable and less expensive than the Nitech reactor that counterbalances partly weaker mass transfer performance.

More generally, it was shown here that the usual technical drawback for operation of these reactions in two-phase systems, i.e., the mastering of the liquid-liquid dispersion, can be circumvented. This is very interesting because such a drawback usually directs the industrialisation of such reactions towards the use of low productivity batch systems using large

agitated tanks with uneasy control of the two-phase dispersion. When efficient continuous operation is really desired, this implies to operate the reaction in a monophasic system in order to be able to use a continuous fixed bed of immobilized enzymes. Additional R&D studies are therefore needed, especially complex immobilisation procedures, with a possible loss of activity. Conversely, using the new intensified reactor technologies proposed here, direct extrapolation to continuous steady operation of a two-phase enzymatic reaction at industrial scale is really possible.

Also, in addition to this interesting result, which is an incentive for promoting industrial enzymatic catalysis, it has been shown that the model reaction chosen here could also be a simple and elegant way to characterize mass transfer performance of these technologies. More precisely, it gives access to comparison of their ability to generate liquid-liquid dispersion and to a global assessment of the generated interfacial area. Therefore, in respect to this criterion, easy benchmarking can unexpectedly be proposed and may constitute an additional tool for the choice of the more suitable technology and optimal design and control of two-phase reactions performed in a continuous mode.

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