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PAPER

Microwave barrel reactor use in trimethylolpropane oleate synthesis by *Candida antarctica* lipase in a biphasic non-solvent process†

Manuel Happe,[a] Pascal Grand,[b] Sébastien Farquet,[b] Sandrine Aeby,[c] Jean-Claude Héritier,[b] François Corthay,[b] Eric Mabillard,[b] Roger Marti,[c] Ennio Vanoli,[c] Alain-François Grogg,[a] Samuel Nussbaum,[d] Alain Roduit,[b] François Tièche,[d] Sam Salem,[e] Carole Constantin,[a] Esther Schmitt,[a] Silvan Zahno,[b] Christoph Ellert,[b] Ahmed Habib,[a] Julien Wyss[b] and Fabian Fischer*[a]

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A novel microwave barrel reactor (MBR) was constructed and used in lipase catalyzed biolubricant synthesis. The MBR is thought as a versatile process tool for biotransformation and green chemistry that overcomes current size limitations in microwave reactors. A lipase mediated biotransformation in the MBR was compared to a state of the art jacketed reactor with external heat exchanger. Oleic acid and trimethylolpropane converted quantitatively (96%) into biolubricants using microwave induction. The heat dissipation in the MBR was analyzed by thermal imaging and inside thermometry. Conversion rates, rate constants and pseudo reaction orders were in line with conventional processing and no microwave effect was detected. The MBR is a versatile new reactor for non solvent, minimal and common solvent processing in the microwave field. While the subject of investigations was biolubricant synthesis in the MBR, the technology described is of wider potential interest in the field of biomass processing and sustainable chemical manufacture.

Introduction

In this work a microwave barrel reactor (MBR) is constructed and tested with a green model reaction, an enzymatic biolubricant synthesis. Biotransformation under minimal and bulk solvent conditions requires novel tools for efficient processing, to transform renewable feed stock into chemicals.[1] Today, the number of industrial biotransformations to convert renewable commodities into bulk chemicals is limited.[2] Corn starch saccharification with thermostable α-amylases is a successful example of such an industrial biotransformation.[3] Acrylonitrile hydrolysis into acrylamide by nitrile hydratase[4] also belongs in this category. Conversely, a whole range of renewable bulk and performance chemicals, like biodiesel, surfactants, polymers and lubricants, are not manufactured by industrial biotransformation as biocatalysis is more expensive than chemical production from mineral oil resources. Therefore, biotransformation is primarily used for the production of value-added speciality chemicals, such as aspartame, nicotinamide, L-carnitine,[5] pharmaceutical intermediates[6] and others.

Alternative reactor concepts are needed for white biotechnology to transform all kinds of biological feedstock into the aforementioned products. Some of these reactors are already widely used, like the membrane reactor for enzymatic catalysis.[7] New reactor designs are being researched, for example for the large scale biodiesel production by algae to improve the open pond approach.[8,9] Microbial electricity production also requires novel microbial fuel cell reactors[10] and bio-hydrogen production in microbial electrolysis cells also demands reactor improvements.[11] Another reactor type is orbital shakers, which are up-scaled for industrial mammalian cell cultivation, recently crossing the 1000 L scale.[12] The combination of a barrel reactor with microwave heating[13] is also such a development that is not described in the literature, according to our knowledge. The resulting MBR promises to be a multipurpose reactor that is not only of interest for biotransformation but for green chemistry in general.

In this work, a microwave barrel reactor (MBR) for eco-efficient biotransformation was constructed to process renewable bulk and speciality chemicals under minimal solvent conditions.[14] Minimal solvent multiphase mixtures are viscous and the barrel type reactor provides adequate mixing options;[15] the rotating reactor tube and the stirrer follow their own programming.[16] Moreover, the barrel design complies well with the particularities of microwave induced heating. Microwaves penetrate dipolar matter only ~3 cm due to an exponential decay.[17] To circumvent this limitation, the reaction mixture can be spread

[a]*Institute of Life Technologies, University of Applied Sciences Western Switzerland, HES-SO ValaisRoute du Rawyl 64, CH-1950 Sion 2, Switzerland. E-mail: fabian.fischer@hevs.ch; Fax: +41 276068615; Tel: +41 276068658*
[b]*Institute of Systems Engineering, University of Applied Sciences Western Switzerland, HES-SO Valais, Sion, Switzerland*
[c]*Institute of Chemistry, University of Applied Sciences Western Switzerland, HES-SO Fribourg, Fribourg, Switzerland*
[d]*Information and Communication Systems Institute, University of Applied Sciences Western Switzerland, HE-arc, St Imier, Switzerland*
[e]*Institute of Economics, University of Applied Sciences Western Switzerland, heig-vd, Yverdon, Switzerland*
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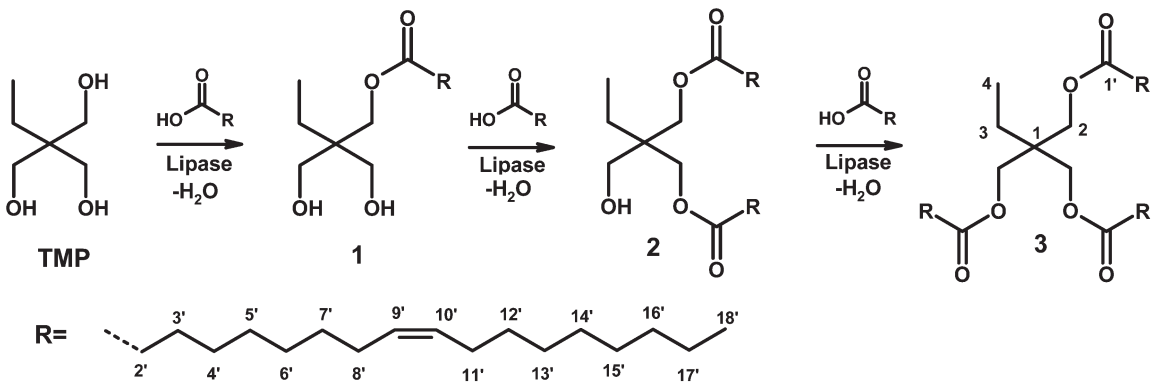

Scheme 1 Irreversible lipase catalyzed biphasic solvent-free synthesis of trimethylolpropane oleates **1–3** from TMP and oleic acid using molecular sieves as desiccant.

over a large surface in the MBR. In contrast, the flow through tube microwave reactors currently used in industrial processing have a limited scale up potential and several reactors are needed to produce as much as within a large scale batch reactor.[18] The MBR design overcomes this limitation as the surface of the reactor tube can theoretically be increased without limits. Therefore, the MBR design is an option to scale-up microwave reactors and facilitate microwave use in chemical and biotransformation processing. Thus, the MBR represents a batch approach for voluminous processing and is a novel solution for chemical engineering. Large-scale microwave reactors are expected to be more energy efficient than conventionally heated alternatives[19] because the heat is conserved in the reactor. Moreover, the MBR is envisioned to be an adaptable tool for low power biotransformation of second generation agricultural commodities into performance and speciality chemicals. In this work, an MBR was constructed and employed for lipase mediated biolubricant synthesis, a typical bioconversion of renewable commodities into bulk products.

Biolubricant synthesis is chosen as a model of a renewable low cost performance chemical. The biodegradability of biolubricants is their key feature in comparison to mineral oil based lubricants as the ecotoxicity[20] is low when lost to the environment. Other important properties are viscosity, low flammability and reduced energy consumption in mechanical applications.[21] The emergence of eco-labels and norms promotes broader use of biolubricants, but most of the time their application is not procured by governments and legislation. The major draw back for biolubricant application is that they are 2–3 times more expensive than petrol based products. Also, the limited choice of speciality biolubricants is a current obstacle to protecting the environment and to ensuring public health by their use. Biodegradable lubricants are, in particular, needed for total loss applications, such as chainsaw use in forestry.[20] Other total losses occur with two stroke motors, some boat engines, but also in mining and tunnel construction. Moreover, lubricants are lost through minor leakages from closed systems by fume exhaust or leaking joints. The performance of biolubricants in automotive applications[22] is in many cases comparable to mineral oil based homologues. Their use in electric cars and numerous small and large electrical machineries is of broad interest. In the medical field, biodegradable lubricants can be used to grease catheters, joints and the like. In conclusion, the number of possible applications appears larger than the number of available biolubricants. Further research is needed to broaden the synthetic possibilities and the application range, including production cost reduction.

The lipase mediated synthesis of trimethylolpropane oleates **1–3** in the microwave field is explored by combining two green chemistry approaches: microwave induction and solvent free biocatalysis.[23] Ester type biolubricants are proposed to replace mineral oil based lubricants.[24] Their lipase mediated synthesis with trimethylolpropane (TMP) and vegetable oils is a white biotechnology approach.[25,26] Chemical catalysts and microwave induction were also examined in biolubricant syntheses[27] and silica-sulphuric acid was as efficient as lipase catalysis, but silica-sulphuric acid yielded brownish by-products.[28] Likewise, different chemical reactions were elaborated to produce various kinds of biolubricants.[29] Beside the often used TMP, neopentyl glycol and pentaerythritol were also esterified using fatty acids or the methyl esters thereof.[30] Lipases maintained their catalytic activity in the microwave field, sustaining temperatures up to 70 °C,[31] also non-solvent lipase mediated catalysis is reported.[32] There are reports about microwave effects in enzymatic catalysis,[33] but none were observed in catalyses with lipases.[34]

To summarize, in this work TMP and oleic acid (OA) were converted into trimethylolpropane oleates **1–3** (Scheme 1) in a microwave barrel reactor constructed for this kind of biotransformation. The process was compared to a jacketed reactor with external heat exchanger.

Results and discussion

The MBR: a novel reactor for green processing

The microwave barrel reactor (MBR) is a tool for bi- and multiphase biotransformation, where non-solvent, minimal or optimal quantities of solvents are used. Barrel reactors are also known as kiln, horizontal tube, or drum reactors. They are used due to their particular mixing options. Their application in biotechnology is of interest in solid phase fermentation, such as wheat straw digestion[35] or mineral bioleaching.[36] The various mixing modes in the MBR are notably different in comparison to stirred tank reactors[15] (Fig. S1†). In addition, the reaction mixture in the MBR is ideally exposed to microwave irradiation as it is

dispersed on the inner tube wall. Low power microwave induction is uncommon in chemical engineering but needed for enzyme catalysis, which requires low temperatures (20–70 °C), to prevent heat induced protein denaturation. To ensure that microwaves reach the reaction mixture the barrel was made of microwave transparent Duran glass (Fig. 1). An internal fibre-optic thermometer provided temperature monitoring and the tube surface was analyzed with a thermal imaging camera.

The mixing modes in barrel reactors without stirrer are described as slumping, rolling/cascading or cateracting.[15,37] The dominance of one of these mixing behaviours depends on the processed material and is easily altered using a horizontal stirrer, such as in the MBR. The stirring arm is removable, but was in general used to ensure heat dissipation. The MBR provides multiple mixing options: the stirrer can be fixed, while the barrel rotates, or the stirrer rotates in the opposite direction to the reactor tube (Fig. S1†). The barrel rotated equally in a centrifugal manner, distributing reaction mixtures as a layer on the inner reactor wall. Such a layer, if not too thick, is irradiated evenly by microwaves, an advantage as microwave intensity exponentially decreases in dipolar liquids or soft matter.[17] In this work the stirrer (17 rpm) and the tube (28 rpm) rotated in opposite directions to homogenize the reaction mixture and to dissipate absorbed heat. All in all, the various mixing modes and the precise power management ensured heat dissipation in the biotransformation mixture.

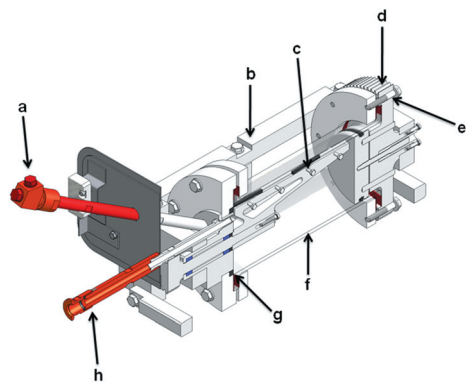

Fig. 1 Reactor cut: (a) service channel, (b) supporting frame, (c) stirring arm, (d) cog-wheel for tube rotation, (e) Teflon™ screw, (f) Duran™ reactor tube of 2 l, length 250 mm, (g) rotational joint, (h) gas inlet.

MBR in solvent-free trimethylolpropane oleate synthesis

The solvent free processing in stirred tank reactors leads to observable mechanical damage of lipase beads and molecular sieves. This is in contrast to the barrel reactor, where such shear forces are reduced and therefore molecular sieves and immobilized enzymes can be used within the reactor tube. Lipase mediated biolubricant synthesis in the MBR is therefore also, from a process technology point of view, a feasible alternative to stirred jacketed reactors. Oleic acid (OA) was converted in the MBR up to 95% (Fig. 2, Table 1). There is some variation in the overall conversions in the MBR and in the stirred reactor, which is due to the total reaction time, that was shown with temperature time screening to find the optimal temperature and duration for quantitative conversions (Fig. 4B). The esterification of TMP and OA in a 1 : 1 or 1 : 2 ratio ($n : n$) led in all cases to a product mixture (**1–3**) as TMP is an achiral triol and lipases exercise no positional selectivity. Using a 1 : 3 ratio with near quantitative TMP esterifications, the triester lubricant **3** was isolated in 68% along with 26% diester **2** and 6% monoester **1** (Table 1, entry 3). In 1 : 3 TMP/OA esterifications the OA conversion depended on diffusion toward the end, visible by a small catalytic constant $k_{rxn} = 0.1\ s^{-1}$. An oleic acid excess would accelerate this third

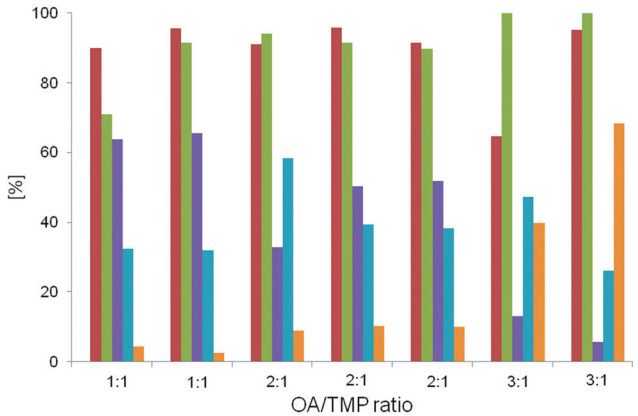

Fig. 2 Scaled-up lipase mediated biolubricant synthesis in the microwave barrel reactor (MBR). Conversions of OA (brown) and TMP (green) and resulting product distributions for different OA/TMP ratios normalized to 100%, monoester **1** (violet), diester **2** (blue), triester **3** (orange).

Table 1 Biotransformation of trimethylolpropane oleates **1–3** from OA, TMP, with molecular sieves and immobilized *Candida antarctica* in the MBR and the jacketed reactor at 70 °C

				Product ratio[b] [mol%]					
Entry	Reactor	TMP:OA Ratio	OA conversion[a] [%]	Mono	Di	Tri	CR[c] [mmol h^{-1}]	k_{rxn} [s^{-1}]	Reaction order n^d
1	MBR	1 : 1	96	65.5	32.0	2.3	36.7	0.46	1.0
2		1 : 2	96	50.3	39.4	10.3	35.2	0.36	1.0
3		1 : 3	95	5.7	26.0	68.4	28.1	0.09	1.1
4	Jacketed reactor	1 : 1	99	59.2	34.2	6.6	32.4	0.23	1.1
5		1 : 2	99	23.5	43.6	32.9	36.6	0.56	0.9
6		1 : 3	94	6.6	23.2	70.2	32.4	0.1	1.1

[a] OA conversion by titration. [b] Product ratio of **1–3** by ^{1}H-NMR. [c] CR (conversion rate) between the second and fifth hour. [d] Rate constants k_{rxn} and pseudo reaction orders n determined by $-r_{OA} = k_{rxn} \cdot C_{OA}^{n}$.

sluggish esterification on TMP.[26] However, an OA excess requires work up and reduces intended eco-efficiency. On the other hand, incomplete esterification of TMP influences the viscosity and is a means to fine tune lubricant properties for specific applications. For analytical purposes, the biolubricants **1–3** were purified by preparative chromatography isolating pure mono **1** and the triester **3**. In contrast, the diester **2** was in all cases obtained as a mixture with near equal quantities of monoester or triester.

The recycling of the immobilized lipase was also examined and the activity after first recycling was almost unchanged. In a third run the reaction time needed to be prolonged to four days to achieve 90% of OA conversion. From this result it is obvious that the enzyme quantity can be reduced at least three times by increasing the reaction time. Catalyst recycling is often considered in the literature but, for example in industrial biotransformation, it is not practised in this repetitive manner.[2a] The enzyme denaturation during recycling is eventually more important than a prolonged use of an adapted quantity in a single use approach, which is also closer to industrial manufacturing.

The energy efficiency of microwave heating *versus* conventional heating is described in the literature[19] but is difficult to show at a lower scale. Process modelling appears most appropriate to estimate energy needs. Such a study showed that if a microwave reactor is well engineered, this heating technology proves more efficient than conventional heating;[38] in addition the transformation efficiency depends also on an equipment factor.[39] In general, larger scale microwave reactors are estimated to be more energy efficient than conventional ones.[19] In longer reactions the microwave heating appears less energy consuming than conventional heating as the heat is applied in pulses on demand and in between no energy is spent for circulating heat exchange fluid. The MBR could equally be reengineered to realize further energy savings. A most notable change would be the magnetron integration into the reactor tube. This is possible for a larger scale MBR and represents a conceptional strategy for 100% energy efficiency. Also to establish a vacuum in the Faraday cage could be a perfect insulation to retain heat in the reactor tube.

For the time being, the lipase mediated biolubricant synthesis in the MBR remains more expensive than mineral oil based lubricant production as energy cost accounts presumably only for ~20%. Raw material cost is more important and is estimated to be ~60% and enzymes ~20%. Nevertheless, the MBR is of interest to produce value added products by enzymatic catalysis, for bioleaching, green chemistry, and for any process that requires smooth stirring.

Heat dissipation in the MBR

Absorbed heat was dissipated by constant mixing and ensured controlled biotransformation. The temperature in jacketed reactors with a heat exchanger is more homogeneous than in the MBR, where microwave power was applied in pulses. The magnetron was electronically switched on and off to adjust the power and therefore the temperature to a desired threshold. As a consequence, the temperature in the reaction mixture dropped slightly between the irradiation pulses. Excessive microwave power or an asymmetric microwave field would lead to superheated zones, also described as hot spots.[40] To prevent such temperature runaways, the process temperature was established over a 10 min interval. A moderate heating gradient, 5 °C min^{-1}, was used, corresponding to 36 W in 15 s long pulses applied twice a minute. To maintain, for example, a process temperature of 70 °C, the irradiation power was reduced to 19 W. The heat dissipation in the reactor barrel was monitored by infrared thermal imaging of the reactor surface (Fig. 3).[41] The histograms of the reactor zone showed temperature differences of up to 7 °C. The reactor tube rotated at 28 rpm and the stirrer at 17 rpm in the opposite direction, to ensure temperature dissipation. An additional test with pure water led to a rather even temperature distribution. For the larger part of the relevant reactor tube the temperature differences were only about 2 °C. A simultaneous measurement with a fibre-optic thermometer on the inside showed that the temperature was ~1.3 °C higher in the barrel than in the hottest area on the reactor tube. The thermo images were recorded under exclusion of heat reflections, visible, for example, in the upper part of the rotating reactor tube as a small horizontal bar (Fig. 3B). This virtually more heated zone persisted even though the reactor tube and stirrer rotated. This kind of artefact is caused by thermo emissions of the hot reaction mixture that is reflected from the ceiling of the Faraday cage serving as containment of the microwave radiation (Fig. S2†). It was found that any object whose temperature differs by >1 °C causes thermo optic effects. In final conclusion, the infrared temperature monitoring of the MBR showed that low power microwave heating facilitates heat dissipation in biphasic non-solvent biotransformation mixtures.

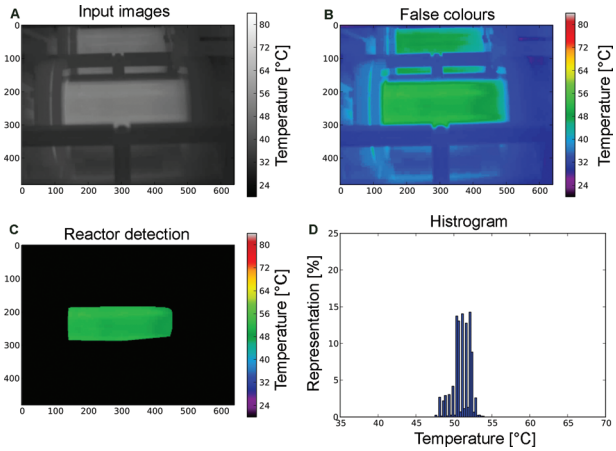

Fig. 3 Heat dissipation in the MBR. (A) Original data in gray scale (B) the coloring, the front part of reactor tube (C) for the calculation of a temperature histogram (D) to examine heat dissipation.

Kinetics in the microwave field

The kinetic characteristics of the MBR process closely matched the ones within the jacketed reactor. The lipase mediated triple step reaction was considered irreversible, A + B → C, A + C → D, and A + D → E (Scheme 1). This was ensured through the use of 3 Å molecular sieves that constantly extracted condensing water from the reaction mixture. Also no transesterification

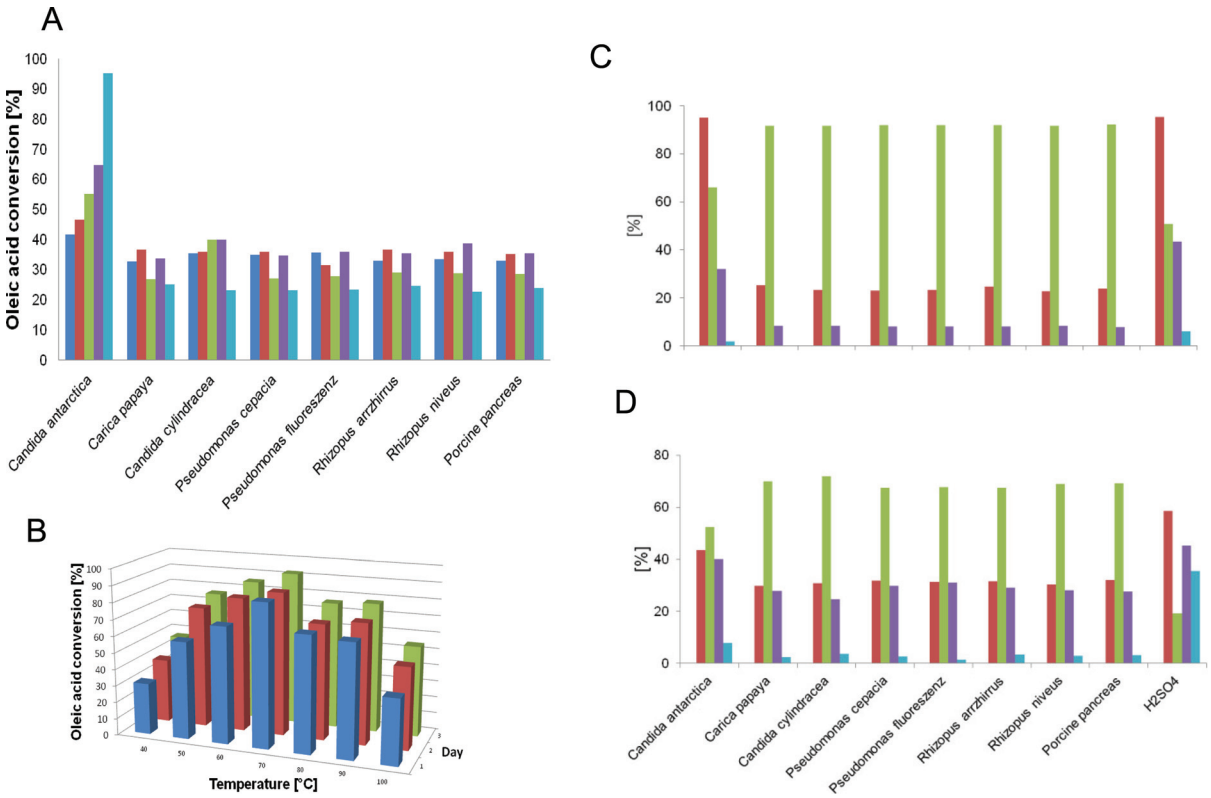

Fig. 4 Lipase screening for various process conditions. (A) Test vials contained 50 U mg^{-1} lipase, 100 mg molecular sieves and stoichiometric ratios of OA and TMP; 1 : 1 (blue); 1 : 0.66 (red); 1 : 0.33 (green). Further more 1 : 1 ratios were combined with 100 U mg^{-1} lipase (violet) and molecular sieve content was subsequently increased 200 mg (light blue). (B) Optimum temperature analysis with immobilized *Candida antarctica* lipase. (C) OA/TMP in 1 : 1 and 1 : 3 ratio in (D), OA conversion (red), and product distribution normalized to 100%, monoester **1** (green), diester **2** (violet) and triester **3** (blue).

activity was observed in the given time frame, as verified by reacting trimethylolpropane trioleate **3** and TMP. Conversion rate (CR) between 28.1 and 36.7 mmol h^{-1} indicated similar reaction rates in both reactors (Table 1). The rate equation, $-r_{OA} = k_{rxn} \cdot C_{OA}{}^{n}$, was applied in order to determine rate constants k_{rxn} and pseudo reaction orders n. The k_{rxn} values, using TMP in a 1 to 2 fold excess, were in the same range and confirmed a similar reaction advancement (Table 1). The reaction orders followed pseudo first order kinetics in all cases, $n = 0.9–1.1$. In quantitative TMP esterification the reaction constants dropped clearly, $k_{rxn} = 0.1\ s^{-1}$, and this was again for both reactors (Table 1, entries 3 and 6). The third sluggish esterification on TMP toward the end of the reaction follows diffusion controlled kinetics.

The mixing in the MBR was expected to be complex, due to the horizontal mixing. Based on the obtained results the process in the MBR was nevertheless yielding results that were in line with the jacketed reactor (Table 1). The kinetic parameters also indicate that there is no microwave effect, which is in line with earlier findings from lipase catalyzed microwave induced heating.[34] Nevertheless, a microwave effect cannot be excluded because studies with hyperthermophilic enzymes showed that such phenomena exist.[33] A more detailed kinetic analysis of OA conversions to elucidate all three reaction constants, k_1, k_2 and k_3, worked to some extent for the conventionally heated process, but appeared challenging to establish for the MBR. This is presumably due to the rather different mixing process in the MBR. Further analyses are needed but appear laborious for the moment as no automatic process analytical technology (PAT) was available for this kind of process. In final conclusion, the kinetic analyses show that the MBR design works equally well as a state of the art jacketed reactor with heat exchanger.

Lipase screening

Eight lipases were screened under various conditions to find the most suited enzyme and the best biotransformation conditions (Fig. 4). Substrate, lipase and molecular sieve quantities were varied. ^{1}H-NMR analyses were made from reaction mixtures to determine substrate conversion, yield and product distribution (Fig. S3†). The screening with 50 U lipase and 100 mg molecular sieves showed that *Candida antarctica* lipase was 10–20% more active than the other seven lipases (Fig. 4A). Doubling the lipase amount (100 U) leaded to ~50% higher conversion for *Candida antarctica* lipase, while the other lipases showed no significant improvements. Using 100 U of *Candida antarctica* lipase and doubling the molecular sieve quantity to 200 mg tripled the OA conversion to 92%. This result shows that *Candida antarctica* lipase is robust and useful, and was therefore chosen for upscale processing in its immobilized form (Fig. 2, Table 1).

The product distribution analysis also showed if lipase exercised size selectivity due to the bulkiness of formed mono **1** and

diesters **2**. Such an effect would justify their application beyond the formerly observed enhanced product purity.[28] Sulphuric acid is known to well catalyse this esterification but leads to brownish products requiring purification. 1 : 1 ratios of OA and TMP yield higher monoester **1** content with lipases than the use of 5% sulphuric acid (Fig. 4C). A similar effect caused by the steric hindrance in the enzyme is achieved with 1 : 3 ratios of TMP/OA. Nevertheless, in 1 : 3 ratios the triester **3** content is rising in all cases when conversions become quantitative (Fig. 4D).

Also a temperature screening was realized with the immobilized *Candida antarctica* lipase showing that 70 °C is the optimal temperature and prolonged reaction allows quantitative conversions. All in all *Candida antarctica* lipase outperformed the other lipases and was therefore chosen for scale-up processing in the MBR.

Analysis

A ^{1}H-NMR based analysis method was developed that provided all conversion information in a simple and rapid procedure (Fig. S3†). An aliquot of the crude reaction mixture was dissolved in THF-d_8 and the directly recorded spectrum showed oleic acid and TMP conversion, lubricant yield, and product distribution **1–3** at once. The triplet of the H-C(2′) protons of the oleic acid shifted from 2.20 to 2.27 ppm upon esterification and was used to calculate oleic acid conversions. The methylene singlet H-C(2) in TMP shifted upon esterification from 3.46 ppm to three different positions, at 3.98 (**2**), 3.99 (**1**), and 4.00 (**3**), yielding the product distribution. An APCI mass spectrum confirmed the presence of three products (Fig. S4†).

Crystallization and fusion points, viscosity, and biodegradability were analyzed as well. The purified fully esterified TMP **3** crystallized (became a solid) in DSC at −70 °C and melted again at −37 °C (Fig. S5†). In comparison, the monoesterified lubricant **1** crystallized at −13 °C and melted at 8 °C because of the higher polarity of the non-acylated hydroxyls in **1**. Biolubricants with free OH-functions (**1–2**) are of interest due to their higher viscosity but also as precursors for the synthesis of task specific lubricants. Shear viscosity differences were found in particular at lower temperature (Fig. S6†). For mono acylated TMP **1** the viscosity at 10 °C was higher, $\gamma_{10\ °C}$ = 0.60 Pas, than with the fully esterified product **3**, $\gamma_{10\ °C}$ = 0.13 Pas. With rising temperature, the viscosity became low and equal, $\gamma_{75\ °C}$ = 0.02 Pas. A biolubricant must be able to sustain adverse conditions during use and nevertheless become biodegradable upon loss to the environment. Several methods allow the determination of the biodegradability, and the ISO-Norm 9439 was employed to examine this property.[42] The anaerobic degradation in aqueous environment was examined with microbes typically found in waste water treatment plants. The fully acylated biolubricant **3** degraded under these conditions to 79.7% in 28 days, outperforming mineral oil based lubricants[43] (Fig. 5).

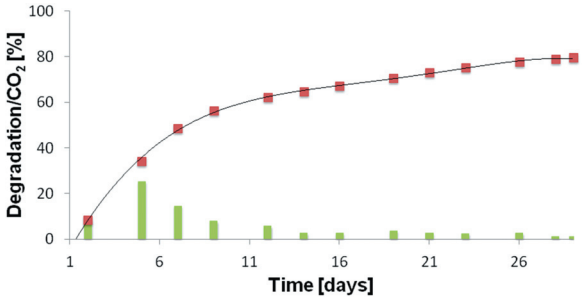

Fig. 5 Biodegradation of trimethylolpropane trioleate **3** (■) and CO_2 evolution (green bars) under anaerobic aqueous conditions.

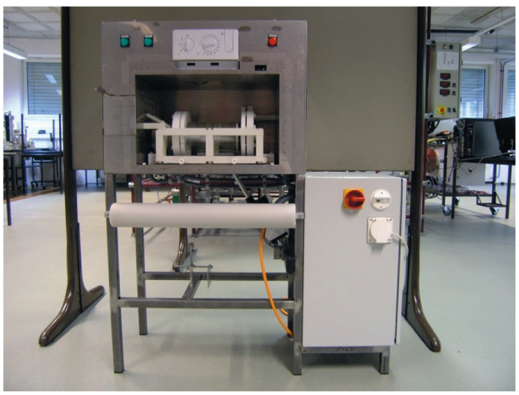

Fig. 6 The microwave barrel reactor with controller box.

Material and methods

Chemicals

Trimethylolpropane (TMP) 98% and oleic acid (OA) (90%) were purchased from Sigma-Aldrich [Buchs, Switzerland]. The acid content in OA was determined by titration. 3 Å molecular sieves [Merck, Darmstadt] were activated before use. The lipase Novozyme 435 was obtained from Novozyme [Denmark]. Acetonitrile was of Lichrosolv Grade [Merck, Darmstadt], ethanol abs., and hexane (isomeric mixture) from Brenntag [Schweizerhall, Basel].

Reactor construction

The MBR was constructed based on a 2 L Duran glass cylinder [Quartz Technique SA, Neuchâtel, Switzerland] (Fig. 1 and 6). The supporting frame, mechanical parts, screws, fittings, and the stirring arm were crafted from Teflon™. The reactor cylinder and the stirrer rotated at variable speeds (1–50 rpm) in either direction. The magnetron and the wave guide were located above the horizontal reactor barrel. Irradiation power and stirring were controlled over the internet with a specifically programmed user interface (web browser) permitting safe remote control through a personal computer connected to the internet (Fig. 7). A handheld microwave detector located potential microwave leakages. The reaction temperature was periodically controlled with an internal fibre-optic thermometer and the heat dissipation in the barrel by thermo imaging.

Procedure for trimethylolpropane oleates 1–3

With the microwave barrel reactor (MBR). 56.50 g (0.200 mol) oleic acid, 26.80 g (0.200 mol) trimethylolpropane (TMP), 9.56 g (95 600 UBT) Novozyme 435, and 26.00 g 3 Å

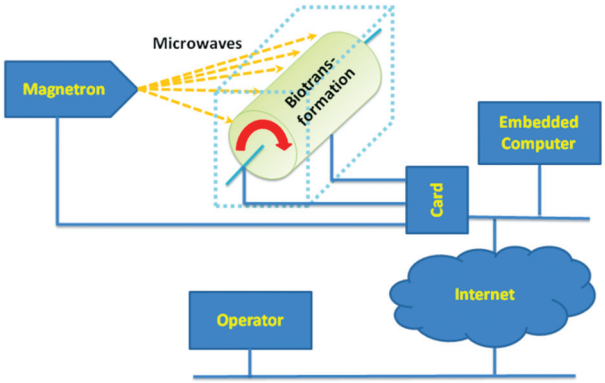

Fig. 7 The microwave barrel reactor (MBR) operated through a web browser over an embedded computer connected to the internet.

molecular sieves were added to the MBR. For 1 : 2 and 1 : 3 stoichiometries, TMP quantities were reduced to 0.132 and 0.066 mol. The reactor tube rotated at 28 rpm and the stirring arm rotated at 17 rpm in opposite direction. Microwave power pulses of 36 W were applied every 30 s for 15 s to heat the reaction mixture up to the processing temperature. It took 10 min (5 °C min^{-1}) to reach 70 °C. The process temperature was maintained by reducing the irradiation power to 19 W. The oleic acid conversion was assayed by titration and the completeness of the transformation was verified by ^{1}H-NMR (THF-d_8). Purification for further analyses was realized by preparative chromatography on silica gel using EtOAc–heptane (1 : 2) as eluent.

With the jacketed reactor. The above described process was also realized in a 500 mL jacketed glass reactor with external heat exchanger and a mechanical stirrer (800 rpm). The heat exchange fluid was heated to 75 °C to ensure a process temperature of 70 °C.

Lipase screening

90, 180 or 270 mg (0.66, 1.33 or 2 mmol) trimethylolpropane, 600 mg (2 mmol) oleic acid (90%), 50 U lipase and 100 mg molecular sieves 3 Å were added to a 10 mL glass vial and sealed. A series of vials were fixed on a revolving cylinder (8 rpm) and processed at 70 °C for three days. Samples of the reaction mixtures were analysed by ^{1}H-NMR (400 MHz) in THF-d_8 to determine conversion and product distribution.

Enzyme recycling

A 200 mL reaction mixture from scale-up processing was diluted with 150 mL hexane and filtered. The filter cake was washed with hexane and suspended in pure water to sediment molecular sieves and recover lipase beads from the supernatant solution. The beads were washed and reused in a next process cycle.

Conversion and yield determination

By titration: 0.5 g reaction mixture was centrifuged (5 min/12 000*g*) and the supernatant oil transferred to a gauged 10 mL flask filled-up with absolute ethanol and titrated with 0.2 M NaOH solution.

By ^{1}H-NMR (400 MHz, THF-d_8): An aliquot of the reaction mixture was dissolved in THF-d_8 and the recorded ^{1}H-NMR (400 MHz) spectrum provided all needed information: substrate conversion, yield, including mono-, di- and triester content read from characteristic H-C(2) singlets at δ_H = 3.99 (**1**), 3.97 (**2**), and 4.00 ppm (**3**).

Heat dissipation

The heat dissipation was examined with a ThermoVision A320 and ThermaCAM E65 [FLIR, Pergam-Suisse AG] thermo camera. For security reasons, microwave irradiation was halted before imaging. Pictures were registered primarily of the barrel rotating at 28 rpm with the stirrer moving in the opposite direction at 17 rpm. The data was recorded in false colours and the obtained high resolution histograms were then transformed into false colour heat distribution information. A specifically developed software was programmed to analyze temperature dissipation in the reaction zone only (Fig. 3).

Microwave power determination

To quantify applied microwave power, the received heat enthalpy in the fully equipped MBR was determined. For this purpose 200 mL water preheated to 70 °C was filled into the MBR tube and the temperature loss was registered during 10 minutes. Subsequently the MBR was cooled to room temperature and 200 mL water of ambient temperature was filled into the empty MBR and irradiated with 50% of available power for 10 minutes (a 15 s long microwave pulse every 30 s). Finally, the heat loss and the absorbed heat enthalpies were combined. The net power pulse reaching the reaction mixture was 36 W (545 J), using 50% of available power.

Lubricant analyses

Crystallization and melting points. They were determined by differential scanning calorimetry (DSC) on a 821^e Mettler TOLEDO calorimeter. The measurement for the crystallization points (solidification) started at 25 °C with a cooling rate of −2 °C min^{-1}. The melting points were determined next, starting at either at −30 or −150 °C with a 1 °C min^{-1} heating gradient.

Viscosity. Shear viscosity was analyzed with a Parr Physica MCR 300 rheometer, at −5 to 90 °C with a constant shear rate of $d\gamma/dt = 5\ s^{-1}$.

Biodegradability. Assessed by anaerobic degradation according to the ISO-Norm 9439,[42] yielding a measurable time dependent CO_2 evolution compared to a reference process.

Structure analysis. ^{1}H and ^{13}C-NMR spectra of purified products were recorded on a Bruker Avance 400 MHz NMR spectrometer using THF-d_8. FT-IR (film) analyses were conducted on a Nicolet 5700 FT-IR from Thermo Electron Corporation and APCI Mass Spectroscopy on a Hewlett Packard Series 1100 MSD.

Trimethylolpropane monooleate 1

δ_H (400 MHz, THF-d_8) 5.36–5.29 (m, 2H, C*H*), 3.99 (s, 2H, CH_2), 3.42 (s, 4H, CH_2), 2.30–2.25 (t, 2H, CH_2), 2.07–2.01 (m, 4H, CH_2), 1.61–1.58 (t, 2H, CH_2), 1.49–1.39 (m, 2H, CH_2), 1.32–1.29 (m, 20H, CH_2), 0.90–0.85 (m, 6H, CH_3).

δ_C (100 MHz, THF-d_8) 173.08(q), 130.41(t), 130.35(t), 65.25(s), 63.76(s), 43.59(q), 41.54(s), 34.50(s), 32.73(s), 30.58(s), 30.54(s), 30.35(s), 30.15(s), 30.11(s), 30.05(s), 29.96(s), 25.71(s), 23.43(s), 23.14(s), 22.89(s), 14.34(p), 7.73(p).

FT-IR (film): 3410 w, 2922 s, 2853 m, 1737 m, 1715 m.

UV-Vis (1 mM in hexane): λ_{max} 220 nm; ε = 682 l mol^{-1} cm^{-1}.

m/*z* (APCI-MS): 399.2 (M − H$^+$, $C_{21}H_{40}O_7$ requires 398.6318).

Refraction index: (product mixture) n_{25} = 1.4708; (pure) n_{25} = 1.4684.

Melting point (DSC): 8 °C.

Crystallization point (DSC): −13 °C.

Trimethylolpropane dioleate 2

δ_H (400 MHz, THF-d_8) 5.36–5.29 (m, 4H, C*H*), 3.97 (s, 4H, CH_2), 3.42 (s, 2H, CH_2), 2.30–2.25 (t, 4H, CH_2), 2.07–2.01 (m, 8H, CH_2), 1.61–1.58 (t, 4H, CH_2), 1.49–1.39 (m, 4H, CH_2), 1.32–1.29 (m, 40H, CH_2), 0.90–0.85 (m, 9H, CH_3).

δ_C (100 MHz, THF-d_8) 173.08(q), 130.41(t), 130.35(t), 64.34(s), 62.14(s), 43.59(q), 41.54(s), 34.50(s), 32.73(s), 30.58(s), 30.54(s), 30.35(s), 30.15(s), 30.11(s), 30.05(s), 29.96(s), 25.71(s), 23.43(s), 23.14(s), 22.89(s), 14.34(p), 7.73(p).

FT-IR (film) (as obtained): 3521 w, 2923 s, 2853 m, 1739 s.

UV-Vis (1 mM in hexane): λ_{max} 220 nm; ε = 682 L mol^{-1} cm^{-1}.

m/*z* (APCI-MS): 663.7 (M − H$^+$, $C_{42}H_{78}O_5$ requires 663.0870).

Refraction index: (product mixture) n_{25} = 1.4708.

Trimethylolpropane trioleate 3

δ_H (400 MHz, THF-d_8) 5.37–5.29 (m, 6H, C*H*), 4.00 (s, 6H, CH_2), 2.30–2.25 (t, 6H, CH_2), 2.06–2.01 (m, 12H, CH_2), 1.61–1.57 (t, 6H, CH_2), 1.49–1.39 (m, 2H, CH_2), 1.32–1.29 (m, 60H, CH_2), 0.90–0.85 (m, 12H, CH_3).

δ_C (100 MHz, THF-d_8) 72.82(q), 130.41(t), 130.36(t), 63.96(s), 41.55(q), 41.54(s), 34.41(s), 32.74(s), 30.59(s), 30.55(s), 30.36(s), 30.16(s), 30.12(s), 30.05(s), 29.97(s), 25.93(s), 23.43(s), 27.90(s), 25.61(s), 23.44(s), 23.44(s), 14.36(p), 7.60 (p).

FT-IR (film): 2921 s, 2852 m, 1738 s.

UV-Vis (1 mM in hexane): λ_{max} 220 nm; ε = 682 l mol^{-1} cm^{-1}.

m/*z* (APCI-MS): 927.8 (M − H$^+$, $C_{60}H_{110}O_{76}$ requires 927.5421).

Refraction index: (product mixture) n_{25} = 1.4700; (pure) n_{25} = 1.4680.

Melting point (DSC): −37 °C.

Crystallization point (DSC): −70 °C.

Conclusions

The newly constructed microwave barrel reactor (MBR) enabled quantitative biolubricant synthesis. The MBR is well adapted to solvent-free or minimal solvent biphasic biotransformation and promises to be a versatile reactor for green chemistry. The mixing options are manifold, enlarging the process options in multiphase viscous mixture processing. Moreover, the MBR design overcomes the size limitation of microwave reactors currently available for chemical process engineering as the design allows a scale-up to large scale microwave batch reactors. The next step is to construct such larger reactors with an enclosed magnetron to become near 100% energy efficient. The heat dissipation in the MBR is optimized through the various available mixing modes. Kinetic parameters from MBR processing, such as rate constants k_{rxn}, reaction orders *n* and conversion rates (CR), matched those within a jacketed reactor with heat exchanger.

The lipase mediated oleic acid and TMP conversion into trimethylolpropane oleates **1–3** was nearly quantitative in all experiments, 94–95%. ^{1}H-NMR using THF-d_8 as solvent allowed rapid quantification of substrate conversion, yield, and product composition in a single recording. Purified biolubricants provided fusion points between 8 °C (**1**) to −37 °C (**3**). The observed variation in the melting points correlated with the number of free hydroxyl groups. From a green perspective, the most important parameter of a green lubricant is its biodegradability. The purified trimethylolpropane trioleate **3** degraded to 79.7% in 28 days.

In final conclusion, the MBR is a means to reduce solvent use and energy consumption in bioconversion and green chemical processing. Biphasic and multiphase reaction mixtures are more easily processed in a barrel reactor than a tank reactor.

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