Counter-currently operated reactive extractor with additively manufactured enzyme carrier structure

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ABSTRACT The development of continuous flow reactors for heterogeneous chemical or biochemical reactions raises the question of efficient mixing and catalyst immobilization. Especially, in those cases where hybrid reactor concepts are aimed at, combining of reaction and extraction in one apparatus requires a solution for reaction and extraction phase distribution and immobilization concepts. Precisely designed structures inserted as reactor packings can be used to control the multiphase hydrodynamics and to act as a catalyst carrier simultaneously. However, the decision for the most suitable structure for specific reaction systems remains a challenge. While numerical simulations with computational fluid dynamics (CFD) has limitations regarding the complex interactions in multiphase flows, performing experiments using rapid prototyping (RP) offers the possibility of a fast fabrication and verification of tailor-made structures for specific flow characteristics, efficient mass transport and high conversion rates. Additionally, RP can be used to quickly approve results from CFD simulations in experiments. In the presented work the development of a counter-currently (CC) operated additively manufactured reactor (AMR) for the decarboxylation of ferulic acid (FA) to 2-methoxy-4-vinylphenol (MVP) along *in situ* extraction with *n*-heptane is shown. Here, the use and optimization of periodic open-cell structures (POCS) as a carrier for the enzyme phenolic acid decarboxylase and a distributor for the extraction phase is targeted. By rapid prototyping of transparent structures and their examination with respect to the induced flow characteristics of colored heptane, a structure could be optimized for the specific reaction system. The additive manufacturing of the POCS and its application in a CC AMR enabled 95% conversion of 5 mM FA in two hours and a MVP concentration in reaction phase below 0.5 mM.

KEYWORDS rapid prototyping, additively manufactured reactor (AMR), periodic open-cell structures (POCS), in situ product removal (ISPR), biocatalysis

INTRODUCTION

Enzyme catalysis plays a major role in industrial production and processing of pharmaceuticals, food, detergents, biofuels, paper and pulp. It represents an environmentally benign alternative to chemical synthesis routes in respect to minimize waste production because of often showing a higher chemo-, regio- and stereoselectivity than their chemical counter parts leading to a considerable attraction in catalysis.¹ Commercialization of biocatalysis is projected to further increasing in the coming years² due to the facilitation of much more competitive processes compared to chemical catalysis.³ However, the efficiency of enzymatic catalysis can be hampered due to a limited reaction speed⁴, limited stability or effects of product inhibition.⁵ For instance, the decarboxylation of the nature abundant ferulic acid (FA) to the valuable⁶ 2-methoxy-4-vinylphenol (MVP), being used as 'smoke' aroma in food industry, is catalyzed by the enzyme phenolic acid decarboxylase (PAD) from *Mycobacterium colombiense* and shows a strong competitive inhibition already at low product concentrations ($K_i = 0.14 \pm 0.04$ mM).⁵ Several process solutions are reducing product inhibitions via *in situ* product removal (ISPR). Beside evaporation, membrane dialysis, reversible complex formation, product ad- and absorption or stripping, the *in situ* extraction of inhibiting products is one convenient way of increasing the process efficiency.^{7,8} Various studies have shown an enhancement in reaction efficiency when implementing the *in situ* extraction of MVP with different solvents.^{5,9-12} Though, those extractions have rarely been optimized in respect to the mass transport efficiency. One commonly used extraction method is counter-current (CC) extraction with the advantages of continuously high concentration gradients compared to co-current extractions. This directly influences extraction rates since mass transport of solutes to and through interfaces is driven by convective mass transport and concentration gradient depending diffusion through laminar boundary layer. Beside the choice for a counter-current flow mode, extraction-influencing parameters can be

controlled by internal packings to guide the flow of both phases. The change in free cross-sectional area in flow direction within a packing induces accelerations and decelerations, the braking of flow structures and by that the enhancement of mixing on micro- and macroscale. The breakup of droplets can be controlled by the packing itself or by the induced shear forces to increase the interfacial area between reaction and extraction phase. The droplet rising velocity distribution is influencing the hold up and residence time of the individual phases.^{13,14} However, in the case of free biocatalysts being used in continuous CC extraction for an intensified ISPR several challenges arise: they are less stable and their presence can lead to formations of emulsions due to their surface activity¹⁵. In contrast, these hindrances can be overcome by enzyme immobilization.^{16,17} In order to combine immobilized enzymes with ISPR in a CC flow reactor, the selection of a packing material and structure is crucial. The chosen packing needs to work as an enzyme carrier and an extraction phase distributor at the same time. Relevant characteristics such as the chemical and mechanical stability of the material needs to be considered.¹⁸ Likewise, the specific surface properties of a chosen material as well as its absolute surface area, determines the amount of enzyme activity, applied per reaction volume.

Via the additive manufacturing (AM) of distinct packings, a broad variety of structure characteristics can be designed, fulfilling the specific demands of an efficient ISPR. Various materials can be used for the fast manufacturing of packing structures at low costs and with less waste of material. The flow behavior of an organic extraction phase can directly be influenced by the precise geometric design of packing structures. For instance, in 2017, Möller *et al.* have shown, that there is a great potential of additively manufactured periodic open-cell structures (POCS) concerning energy efficiency and mass transfer performance compared to commercially used structured packings¹⁹. POCS have been investigated concerning their potential of enhanced heat conduction in catalytic fixed bed reactors before.²⁰ In addition, POCS were implemented for

process intensification purposes in trickle bed applications.²¹ With the help of the fast production of 3D structures for test purposes via AM, called rapid prototyping (RP), POCS designs can be tested rapidly regarding their anticipated inductions of specific flow behaviors.

Enzyme performance can be enhanced by their use in flow reactors²². In this work, the concept of an additively manufactured reactor (AMR) as a flow reactor is presented by the biocatalytic synthesis of MVP with PAD as an immobilized biocatalyst as a model reaction. To that end, the workflow for designing, fabrication, evaluating and iteratively optimizing POCS to be used as an enzyme carrier and distributor for a heptane extraction phase in a CC reactive extractor is outlined exemplary.

RESULTS AND DISCUSSION

The workflow for the design and selection of POCS, the analysis of POCS induced extraction phase flows and the MVP synthesis with ISPR within a hybrid CC AMR equipped with POCS is presented (Figure 1) and will be the chronology of following sections.

Figure 1. Systematic, iterative optimization of distributor - carrier packings. CAD = computer-aided design; AMR = additively manufactured reactor

Design of structures

The structures investigated show different performance with respect to radial distribution, residence time and provided specific surface area. On the one hand the structure can be used for enzyme immobilization on their surface, on the other hand to enhance the interfacial surface area between extraction and reaction phase by splitting the extraction phase into smaller droplets. All investigated structures consist of a cubic unit cell, which was scaled in CAD in all three spatial directions and adapted to the reactor geometry. The structures are called periodic open-cell structures (POCS). Geometrically, they are primarily defined by the unit cell shape, the cell size

and the web diameter. Preliminary tests have shown that a very dense packing with a high specific surface area has led to an accumulation of the extraction phase within the structure under the selected flow conditions (Figure S1). For this reason, coarser structures were selected, which proved useful in subsequent experiments, as the coarser cells with a web thickness of 1 mm cut the droplets into smaller, thereby increasing the specific surface area for extraction and thus the extraction performance.

The structures according to Table 1 have been selected to achieve the following performance:

- Structure 1: high radial distribution and large droplet sizes due to the comparatively large unit cell size.
- Structure 2: high radial distribution, but even finer droplet sizes due to a smaller unit cell size compared to structure 1 and thus a possibly higher extraction performance. In addition, more specific surface area is made available for enzyme immobilization due to a denser structure design.
- Structure 3: Same unit cell size as structure 2 but longer residence times due to the meandering flow as a result of the aperiodic open-cell structure (APOCS). The APOCS also has the comparatively highest surface area for enzyme immobilization.

Table 1. Packing characteristics applied as extraction phase distributors

description	Structure 1	Structure 2	Structure 3
computer-aided design (CAD)
top-view cross section
unit cell size s^* [mm]	6	5	5
web thickness [mm]	1.0	1.0	1.0
porosity [-]	0.94	0.91	0.91
surface [mm ²]	10230	14930	14032
solid volume [mm ³]	2579	3854	3592
void volume [mm ³]	42660	41385	36169
surface/reaction volume [mm ⁻¹]	0.24	0.36	0.39

* s

Rapid Prototyping of periodic open-cell structures

In this work, rapid prototyping has been used for the selection of most suitable structure acting as enzyme carrier and extraction phase distributor in a hybrid reactor for an efficient MVP synthesis. To that purpose, prototypes out of a transparent resin have been implemented in an evaluation system. Their ability to distribute heptane colored with sudan red 7B as an extraction phase within a CC two-phase reactor setup was tested by the optical analysis of the resulting flow behavior.

However, the application of packing in the example of MVP synthesis requires a manufactured material, which is chemically resistant against the organic solvent and has suitable properties for the immobilization of PAD, which was not shown for acrylic resin. Preliminary studies have shown one immobilization method for the adhesive immobilization on the additively manufacturable material polyethylene terephthalate (PET) via the genetic fusion of PAD with so called anchor peptides. Immobilisates of this type can be used for the synthesis of MVP from FA

in a buffer-heptane two-phase system.²³ The universal applicability of one anchor to different materials with similar properties has been shown by Dedisch *et al.*²⁴. Manufacturing POCS out of PET with fused deposition modelling (FDM) is limited by the resolution and effects like string formation between unit cells during the manufacturing process are affecting the optimized flow of heptane. Therefore, structures being used in the AMR have been manufactured out of polyamide 12 (PA12) via selective laser sintering (SLS), since this material offers a possibility of high manufacturing resolution (50/1-2 μm ; XY/Z)²⁵, is chemically resistant against heptane and was assumed suitable for an adhesive immobilization with anchor peptides. Additionally, SLS approach offers high surface roughness beneficial for enzyme immobilization.²⁶ The difference in the surface and accuracy of one structure depending on the manufacturing technology and its material, is depicted in Figure 2.

Figure 2. Top view of periodic open-cell structures (structure 2, cf. Table 1): A) computer-aided design (CAD); B) additively manufactured out of photo reactive resin (methyl methacrylate + photo initiator) with stereo lithography (SLA); C) additively manufactured out of polyethylene terephthalate with fused deposition modelling (FDM); D) additively manufactured PA12 with selective laser sintering (SLS)

Compared to FDM, SLS offers high accuracy and reproducibility (Figure 2) not only for manufacturing of POCS with web thicknesses of 1 mm (shown here) but also down to 0.6 mm, with specific parameters given in experimental section. Another benefit of SLS is the preheating

of the powder, which allows the control of thermal gradients and thus eliminates the need for support structures (preheated powder acts as support itself). However, with preheating of powder its removing out of the structure post manufacturing becomes challenging for fine structures, as the powder bonds together gently. For this reason, when designing POCS for SLS, it is necessary to ensure that the grid structure is sufficiently rigid to allow the removal of the residual powder.

Flow analysis

Colored heptane ($67 \mu\text{g mL}^{-1}$ sudan red corresponding to $0,168 \text{ mM}$) was recorded while flowing through transparent structures 1-3 (cf. Table 1) within a CC AMR (Figure 3). The formed spherical and elliptical droplets were analyzed regarding their size distribution (Figure 4) by measuring their major **a** and minor **b** diameter and calculating an equivalent spherical diameter (ESD) according to formula 1. By analyzing three frames per structure a mean value and standard deviation was calculated.

$$ESD = \sqrt[3]{8 \cdot a^2 \cdot b} \quad (1)$$

The volume of all droplets within one reactor **V**, their formed interfacial area **A** and the surface to volume ratio is depicted in Table 2. It is worth mentioned that effects of light scattering and contortions due to a round reactor shape were not considered. To get an idea about the residence time distribution of heptane volumes in CC AMR with three different structures, the colored heptane was pumped out with uncolored heptane. The light intensity of recorded frames during this procedure was changing dependent on the amount of color in the reactor. The less color is present, the less light is absorbed, and the higher is the recorded intensity of video frames. This intensity, dependent on the time of discoloration, is depicted in Figure 5.

Figure 3. Flow analysis of colored heptane in a counter-current reactor with different structures (cf. Table 1) as distributor. Volume flow rate aqueous phase (0.5 M KPi , pH7 buffer) = 32 mL min^{-1} , flow rate organic phase (colorized *n*-heptane) = 32 mL min^{-1} , temperature: 37 °C. Black arrows indicating iterative redesigns.

Figure 4. Mean droplet size distribution of colored heptane within periodic open-cell structures.

Three frames each were used for measuring droplet sizes and building standard deviations, indicated by error bars.

Table 2. Summarized droplet number, Volume, Area and ratio of all droplets within investigated POCS, mean values of biological triplicates

structure	$\sum \text{droplets [-]}$	$V [\text{cm}^3]$	$A [\text{cm}^2]$	$A/V [\text{cm}^{-1}]$
1	87 ± 3	20.4 ± 6.2	131 ± 35	6.5 ± 0.3
2	131 ± 11	13.9 ± 4.3	124 ± 34	9.0 ± 0.4
3	101 ± 4	15.7 ± 3.3	111 ± 12	7.3 ± 1.3

Figure 5. Normalized intensity during discoloration: Visualization of colored heptane residence time in a CC AMR with internal packing structure 1 - 3 to be found in Table 1. Complete discoloration was obtained after 15 min (900s) for every structure.

Structure 1, with a cell diameter of 6 mm, a surface to volume ratio of $0.24 \text{ mm}^2 \text{ mm}^{-3}$ and a porosity of 0.94, induces the breakup and dispersion of heptane droplets (Figure 3). A distribution across the diameter and fast moving droplets is observed. This fast movement leads to small residence time of heptane volumes (80% discoloration after 25 s, Figure 5). This is in accordance with a high share of faster moving big droplets (Figure 4). The high share of big droplets leads to a small surface to volume ratio of $6.5 \pm 0.3 \text{ cm}^{-1}$, inefficient for an extraction. To generate smaller droplet sizes and thereby increase interfacial area between aqueous reaction- and organic extraction phase, the unit cell size was decreased to 5 mm. The resulting structure 2 with a higher surface to volume ratio of $0.36 \text{ mm}^2 \text{ mm}^{-3}$ and a decreased porosity of 0.91 (Table 1) causes a higher flow resistance and the generation of more droplets per reactor volume (131 ± 11 compared to 87 ± 3 for structure 1) with a higher share of small droplets (Figure 4). By that the retention time is increased (80% discoloration after 80 s, Figure 5) and a higher area to volume ratio of $9.0 \pm 0.4 \text{ cm}^{-1}$ (cf. Table 2) is obtained. The effect of inserting aperiodicity into the POCS

was investigated with structure 3 with a surface per reaction volume of $0.39 \text{ mm}^2 \text{ mm}^{-3}$ and a porosity of 0.91. Aperiodic parts in the structure aim to increase turbulence in the two-phase system. Within this structure, a breakup of droplets and a fast movement through the structure is observed. Simultaneously, regions in the structure are only entered by few volumes of heptane and not in high exchange (Figure 3). The smaller share of small droplets (compared to structure 2) causes a smaller surface to heptane volume ratio of $7.3 \pm 1.3 \text{ cm}^{-1}$. Due to mentioned dead zones, a long residence time of heptane volumes is observed (80% discoloration after 120 s, Figure 5). To prevent a product MVP accumulation within the heptane phase in the hybrid reactor, shorter retention time, a radial equal distribution and higher interface to volume ratio was targeted. Therefore, Structure 2 with the ability to generate the highest ratio of interfacial area to extraction phase volume ($9.0 \pm 0.4 \text{ cm}^{-1}$), was chosen for being tested in an actual reactive extractor.

Counter-currently operated additively manufactured reactor (AMR)

Fusion proteins, consisting of PAD, a spacer and an adhesion promotor, namely PAD-DSI-GS, has been immobilized according to [23] on the PA12 structure followed by a rinsing step. Subsequently, the decarboxylation of ferulic acid in a CC AMR has been realized (Figure 6). The recycling of heptane has been performed by distillation to enable a small product concentration within the heptane phase in the AMR.

Figure 6. Process scheme (left) and experimental setup (right) for the decarboxylation of ferulic acid within an additively manufactured reactor (AMR) and direct solvent recycling. Aqueous phase volume: 70.5 mL; organic phase volume system: 235 mL; 1: thermostat (37 °C), 2: oil bath (140 °C), 3: water cooling (10 °C), 4: switch valve, 5: heptane reservoir, 6: reactive packing (structure 2) as distributor and catalyst carrier. 7,8 pump rates: 32 mL min⁻¹.

Within the reactor, a MVP concentration below 0.3 mM in the organic and below 0.5 mM in aqueous phase during the reaction was examined (Figure 7).

Figure 7. FA and MVP concentrations in 56 mL aqueous phase with 4.8 mM FA starting concentration in 0.5 M KP_i buffer (pH 7) and 12 mL (235 mL in whole system) organic phase (OP) within a CC AMR ($T = 37^\circ\text{C}$); Packing structure 2; material: PA12 (manufactured with SLS); immobilization conditions for PAD construct DSI-GS-PAD²³: 0.18 mg mL^{-1} corresponding to 4.5 U mL^{-1} in 100 mL KP_i buffer at 4°C for 10 min.

Using an initial FA concentration of 4.8 mM, the maximum product concentration in organic phase of 0.5 mM was observed after 15 min. Within this time 61% of the FA was converted, which would have resulted in a MVP concentration of 2.9 mM without ISPR. Consequently, heptane, dispersed by AM POCS, extracted 83% of the produced MVP *in situ* in the first 15 min. Naturally, the MVP accumulation in aqueous phase not only depends on the extraction speed but also on enzyme activity and substrate concentration. However, a remaining small product concentration in the aqueous phase is a clear sign for an efficient mass transport between both phases. A fast decrease of substrate concentration was observed in the first hour of reaction (85% conversion), indicating a fast reaction with a limited product inhibition. A FA concentration below 0.7 mM after one hour slows the reaction speed according to Michaelis-Menten kinetics.

The MVP synthesis shown in Figure 7 was conducted after an immobilization and a rinsing with buffer and FA solution (280 mL). In this trial, parts of PAD were retained in the reactor not only due to their adhesive binding on the structure but most likely were also not flushed out due to inconvenient radial distribution in on-phasic mode. When the reactor is emptied before the rinsing step, retained enzyme solution volumes can be prevented. The following Figure 8 shows the AMR performance for one-phasic and bi-phasic MVP synthesis with ISPR in CC mode, when immobilization solution is completely pumped out of the reactor before rinsing. Relative activities were calculated based on the slope of the progression of conversion.

Figure 8. Relative enzyme activity (activity per respective initial activity) within an AMR with (red: 4.9 mM FA in 68 mL) and without (black: 5.1 mM FA in 56 mL and 12 mL heptane) ISPR at 37 °C in 0.5 M KPi buffer (pH 7). Identical PAD immobilization procedure with 0.03 mg mL^{-1} corresponding to 2.0 U mL^{-1} in 100 mL KPi buffer at 4 °C for 10 min, reactor emptied before rinsing. Packing structure 2; material: PA12 (manufactured with SLS).

The conversion plot and the relative activities of both batch experiments (Figure 8) show the benefit of ISPR within an AMR. The activity of immobilized PAD decreases less with ISPR than without due to a reduction of product inhibition. However, PAD is irreversibly inhibited or deactivated by the presence of product in both cases, indicated by the reached conversion of 10 or 18%, respectively. This deactivation was previously described by Pesci *et al.* who advised PAD ($\mu\text{g mL}^{-1}$) to FA (mM) ratios of 6 for high conversions⁵, which could not be reached with limited surface of PA12 packing. Immobilization of more enzymes needs to be realized by enhancing specific surface of manufactured matrix material.

CONCLUSION

This work has convincingly demonstrated that additively manufactured and well-structured enzyme matrices are an effective approach in order to control flow patterns of a multiphase counter-current (bio) reactor. By iterative modifications, an optimized and tailor-made structure for the efficient enzymatic decarboxylation of ferulic acid could be identified, acting as immobilization matrix and extraction phase distributor at the same time. The evaluation of additively manufactured periodic open-cell structures out of transparent resin and their influences on phase behaviors could be simply accessed by a colored heptane phase and camera recordings. The application of optimized structure (manufactured out of PA12) in a reactive extraction system enabled the efficient *in situ* product removal within the synthesis of MVP and therefore lead to overcome product inhibition limitations of the chosen model reaction. We hope that the presented approach could lay a foundation for future and innovative reactor design *en route* to optimized and tailor-made hybrid flow reactors.

EXPERIMENTAL SECTION

General

Acetonitrile (99.95%), ferulic acid (99%), dipotassium hydrogen phosphate (99%), potassium dihydrogen orthophosphate (99%), *n*-heptane ($\geq 99\%$), Luria Bertani medium, and trifluoroacetic acid (99.9%) were purchased from Carl Roth GmbH (Karlsruhe, Germany) and used as received. Sudan red 7B (dye content 95%) was purchased from Sigma-Aldrich Chemie GmbH (St. Louis, USA).

Manufacturing of periodic open-cell structures

Transparent POCS have been manufactured with Form 2 from Formlabs (Somerville, USA) with Clear V4 Resin (Formlabs, Somerville, USA) and a layer thickness of 50 μm .

The POCS produced by selective laser sintering were manufactured with the EOS Eosint P396 machine and the standard polymer powder EOS PA2200 from EOS (Krailling, Germany). A layer thickness of 120 μm was chosen.

Flow characteristics

For tracking the flow of heptane within a CC AMR, transparent POCS were inserted into a glass tube reactor (GE Healthcare GmbH, Frankfurt, Germany). Heptane was colored with sudan red 7B ($67 \mu\text{g mL}^{-1}$) and pumped with 32 mL min^{-1} (ISMATEC® IPC 8, Cole-Parmer GmbH, Wertheim, Germany) CC to aqueous phase (potassium phosphate buffer 0.5 M; 32 mL min^{-1}) The temperature of the heating jacket was set to 37 °C. A light-emitting diodes (LED) panel has been installed behind the reactor, whereas a complementary metal-oxide-semiconductor (CMOS) sensor (DSC-HX400V, Sony) was fixed on a stand and adjusted (ISO value: 100, aperture 8.0,

shutter speed: 1/200 s, resolution 28 MP) for recording the moving heptane phase, which was started after equilibration of the system (20 min after starting the pumps). After 2 min the discoloration was started by switching valve 1 and 2 (Figure 9) and pumping heptane through the reactor into a waste container. Recorded video files were also evaluated via grey value analysis (SI).

Figure 9. Schematic drawing of experimental set up for the analysis of colored heptane phase flow distribution. Colored heptane is pumped counter-currently to the aqueous phase (32 mL min^{-1} each) while flow patterns are recorded by CMOS sensor. For initializing discoloration valve 2 and 1 are switched to pump uncolored heptane through the reactor into the waste.

Counter-current additively manufactured reactor setup

Dimensions and reactor setup

The glass reactor tube with heat jacket (GE Healthcare GmbH, Frankfurt, Germany) had an inner diameter of 26 mm and a height of 200 mm. Inserted additively manufactured packings had a height of 10 mm and an outer wall precisely fitted to the glass tube. Unfilled zones of the glass

reactor were used as a settling zone of respective phases. Individual spacers (50 mm high) were manufactured (FDM) out of poly lactic acid (PLA) and used to fix the position of POCS.

Immobilization

For the immobilization of PAD-GS-DSI on the inserted packing (the production of the fusion protein is described in Büscher et al.²³), the reactor was cooled to 4 °C. 8 mL cell free extract (CFE) (56.1 U mL⁻¹) were added in the reservoir (Figure 6: 5) and mixed with 92 mL potassium phosphate (KP_i) buffer (pH 7, 0.5 M). The whole solution was then pumped for 10 min in a cycle with a flow rate of 8 mL min⁻¹ to allow homogeneous distribution and efficient immobilization of the constructs. Subsequently, the reactor was rinsed with 140 mL of buffer in order to remove unbound enzymes followed by the rinsing with 140 mL FA solution (5 mM FA in 0.5 M KP_i buffer, pH 7). An alternative rinsing approach was investigated: Before rinsing with 140 mL buffer, the reactor was completely emptied. Also after buffer rinsing, the reactor was completely emptied to prevent retained volumes within POCS.

Reaction

While the reaction solution was pumped in a cycle, heptane was pumped through the reactor and collected in a waste beaker. When only clear heptane without aqueous phase was observed in the reactor outlet, the heptane cycle was closed and reaction accelerated by heating the reactor to 37 °C. Aqueous samples during the reaction were withdrawn via a bubble trap through a septum and organic phase at the outlet behind valve 4. Samples were prepared for HPLC and photometer analytics, respectively.

Heptane recycling

Prior to immobilization, the distillation flask was filled with 200 mL heptane and heated to 140 °C with an oil bath. A condenser was cooled with 10 °C water. After 30 min, the first condensed heptane emitted the condenser and was connected to the heptane reservoir. With help of valve 4 (Figure 6) the heptane level in the distillation flask was kept constant.

Concentration measurements

The measurement of FA concentration was conducted via high-pressure liquid chromatography (Method description and sample preparation in SI). The determination of product concentration MVP has been done with the spectrometer UV-1280 from Shimadzu Europa GmbH. Calibration regressions for the concentration measurements of each compound can be found in SI Figure S4, S6 and S7.

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ABBREVIATIONS

AM, additive manufacturing; AMR, additively manufactured reactor; APOCS, aperiodic open-cell structure; CC, counter-current; CFD, computational fluid dynamics; CFE, cell free extract; DSI, Dermaseptin FA, ferulic acid; FDM, fused deposition modelling; HD, hydrodynamics; HPLC, High pressure liquid chromatography; ISPR, in situ product removal; KPi, potassium phosphate; LED, light-emitting diodes; MVP, 2-methoxy-4-vinyl-phenol; PAD, phenolic acid decarboxylase; PET, polyethylene terephthalate; POCS, periodic open-cell structure; PLA, poly lactic acid; RP, rapid prototyping; SLA, stereolithography; SLS, selective laser sintering

SUPPORTING INFORMATION

In the SI, preliminary studies of heptane flows, radial heptane distribution and residence time can be found. Furthermore, analytics of aqueous samples with HPLC and organic samples with spectrometer is described with corresponding calibrations.

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