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EDITOR-IN-CHIEF'S WORD

Micro process engineering is a growing area of the technological process research today, particularly due to the use of experimental systems such as micro structured reactors, or microreactors.

Bruno Zelić, a prominent full member of Croatian Academy of Engineering and Professor at the Faculty of Chemical Engineering and Technology, the University of Zagreb, and his research team have deeply entered the mentioned field by doing laboratory and academic researches. In this issue, as a guest editor, Prof. Bruno Zelić presents some of the significant results achieved in some of his projects.

I believe that you will read this paper with great interest, gaining new insights in this field.

Editor-in-Chief
Vladimir Andročec, President of the Croatian Academy of Engineering

EDITOR'S WORD

Dear readers,

As an academy of engineering sciences, Croatian Academy of Engineering is especially pleased to report on excellent research activities that – beside publications in scientific journals – result in successful collaboration with high-tech oriented industry.

In the same vein, in this issue of Engineering Power we present Guest-Editor Bruno Zelić, Full Member of the Academy and Professor at the Faculty of Chemical Engineering and Technology, and his team.

I am sure you will enjoy reading the presented contributions.

Editor
Zdravko Terze, Vice-President of the Croatian Academy of Engineering

FOREWORD

Microreactors are widely used in different fields of chemical and pharmaceutical industry, biotechnology and medicine. A large surface area to volume ratio, a short diffusion time, a quick and efficient heat and mass transfer are only some of the most important advantages of microreactor systems that have been successfully utilised in the field of chemical synthesis. By using microreactor systems for chemical syntheses, higher conversions and productivity were obtained compared to the reactions carried out in conventional reactor systems. The application of different microreactors for intensification of the chemical and biochemical production processes is intensively studied. According to literature more than 50% of reactions in the fine chemical or pharmaceutical industry could be intensified by microreactor technology. Although a great majority of the reaction systems that are studied in microreactors are connected with chemical synthesis, biocatalysis and biotransformations in a microreactor are demonstrated as promising alternative. Syntheses, oxidations, transesterifications, polymerizations, hydrolyses and coenzyme regenerations catalysed by enzymes or whole cells are just some of numerous different biotransformations that have been successfully performed in microreactors.

The following papers are part of the scientific research in the field of microreactors and microdevices performed by the members of the Laboratory for Bioseparation Processes at the Faculty of Chemical Engineering and Technology, University of Zagreb and their associates from University of Zagreb and J.J. Strossmayer Unniversity of Osijek. Process development, innovations and investigations shown in those papers are mainly supported by Croatian Science Foundation and European Regional Development Fund. The first paper is an extensive overview of microtechnology and microreactors in general. Development of integrated microsystem for production of green note chemical, hexanal is presented in the second paper. The third paper deals with sustainable biodiesel production on micro-scale. All experimental studies performed in microreactors and microdevices are usually supported with mathematical models. Therefore, the fourth paper describes basic principles for mathematical description of hydrodynamics, mass and heat transfer in microreactor systems. Synergy of solid-state fermentation and microreactors is presented in the fifth paper. Finally, the last paper deals with production of microreactor systems by additive manufacturing technology.

Guest-Editor
Bruno Zelić, University of Zagreb, Faculty of Chemical Engineering and Technology

CONTENT

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The Smaller, The Better – Microtechnology for a Macroresults

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Abstract

There is a well-known expression The Bigger, The Better, on which many would agree but when it comes to microreactors it is necessary to make the slight modification and say The Smaller, The Better. Reduction in reactor size emerged in many positive effects on many chemical and biochemical reactions. Faster reactions, smaller usage of reaction components, smaller amount of waste streams, safer reaction conditions, easier process manipulation etc. are just some of the advantages of microreactors. The aim of this review is to present microreactor technology in a simple way and to show its basic characteristics such as structure, advantages and disadvantages, types and general application.

Keywords: *microreactors, production, application, advantages, disadvantages*

1. Microreactors at a glance

When something is present in the science for almost half of the century it can no longer be called as new technology. However, "new" is usually an adjective related to microstructured devices. On the other hand, if we cannot talk about new technology itself, we can still relate the word new to the new concepts, new approaches and new methodologies that are for sure connected with microstructured devices. From the early days, back in 1977 this technology has withdrawn significance attention. From that time, when a microreactor was considered to be a straight tube with microchannel size (Fig. 1a), thanks to the significant scientific and technology progress, the technology itself grown to more complex systems (Fig. 1b).

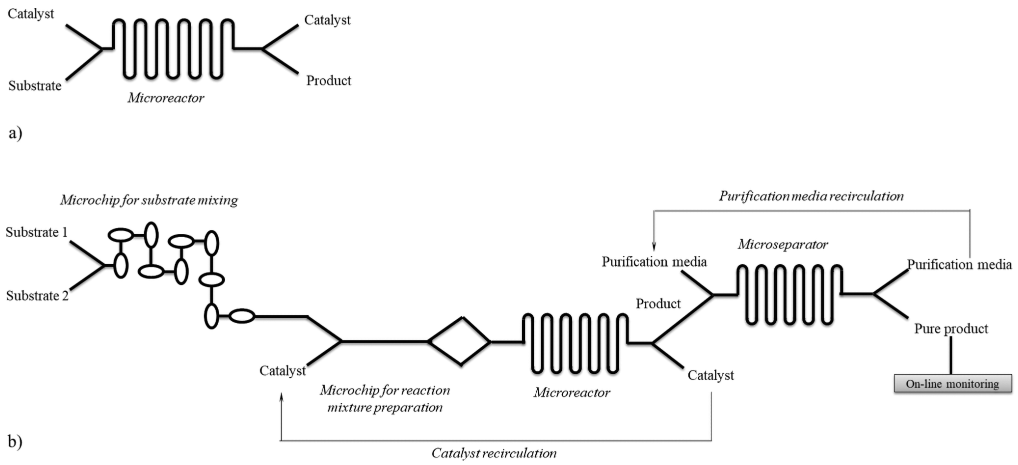

Fig. 1. Schematic diagram of a) simple and b) complex microtechnology system

Regardless to the complexity, microreactors are defined as miniaturized reactor systems constructed by using methods of microtechnology and precision engineering [1] and always consist of same structural units (Fig. 2). First of those structural units is always a microchannel.

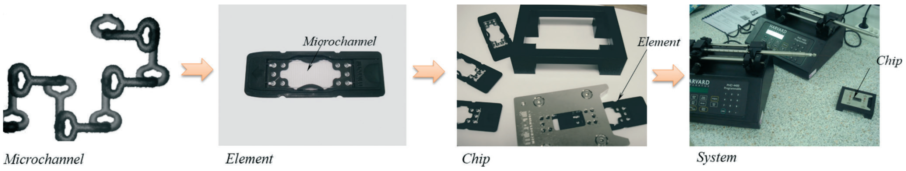

Fig. 2. Structural units of microreactor system

Microreactor is a channel etched in a solid substrate (*element*). As mentioned, *microchannel* is a basic structure of microreactor system but in order to function properly, it needs additional parts (Fig. 2). First, it is an *element*, a material/base in which a channel is positioned. Element and microchannel together form a *chip*. In order to supply microchannel with reaction mixture connection fluid lines are necessary. Together with chip, they form an *unit*. Combination of unit and all other parts of equipment (pumps, systems for analysis etc.) is called a *system* [2]. High pressure pumps are especially important since they are responsible for reaction time determination and flow regulation.

Typical diameters of microchannels are in the range from 10 µm to 500 µm. Microchannels can be in a simple, straight tube form (Fig. 3a and b) or more complex i.e. with integrated micromixers (Fig. 3c and d). Usually, they are round or rectangular in cross section. Depend-ing on a final purposes they are fabricated from different materials mainly glass, silicon, quartz, metals and polymers. The selection of material depends on its chemical compatibility with the reaction mixture and production price. In this way microreactors are adapting to the reaction not *vice versa*. The most commonly used material is glass since it is chemically inert and transparent. Several methods can be applied for etching a microchannel in to solid sub-strate like: micromilling, lithography, embossing processes and laser ablation processing [3]. Choice of method affects channel depth and surface roughness (Fig. 3a and b). Surface roughness is considered to be one of the most important factors for microreactors. Decreasing the size of the channel impact of roughness is significantly increasing and thus increases its impact on reaction. The average channel roughness ranges between 0.8 and 2.5 µm and as mentioned, it depends on microchannel production method [4].

Usually in microchannels with smoot walls, parallel flow is formed and in those with rough walls, segmented flow is more typical. Flow formation is important for several reasons. First, it directly affects the size of interphase area that is important for mass transfer. Second, it affects the phase separation at the exit of microchannel and third, mathematical modelling becomes more challenging when going from parallel to segmented flow.

Chemical and biochemical processes are never simple (Fig. 1b). They are usually a combination of reactant preparation, reaction(s), product separation, reactant recirculation, analysis, etc. As mentioned, this means that microreactor system can vary from a simple one to certainly complex one. Integration of different processes such as, for example, reaction and separation, in microreactor system is currently one of biggest challenges in microreactor technology.

When going on a small scale, size is not the only thing that is changing but also a lot of new physical phenomenon's are observed. They all present a certain challenge to science and usually demand a new approach to reactions that are by now well known on a macro scale. Reducing the scale a lot of new advantages [4] emerged that brought new spotlight on old processes. Microreactors are characterised as safe, reliable, scalable and robust. They allow better process control and offer rapid dynamic response. Some of the other advantages and disadvantages of microreactors are listed in Table 1.

Among all advantages, adaptability of microreactors is considered to be one of the major benefits. This is especially important for process intensification and integration. The best way to simply describe this is to identify microreactors with a LEGO system. Like them, connecting different microstructured devices complex systems can be built up. As mentioned in Table 1., under the Numbering up part, all the rules, all the chemistry that is defined on a single microreactor unit just replicates on all other units when they are multiplied in order to enhance the production capacity. This makes the transition from laboratory to the industry easier and makes the gap between them significantly smaller.

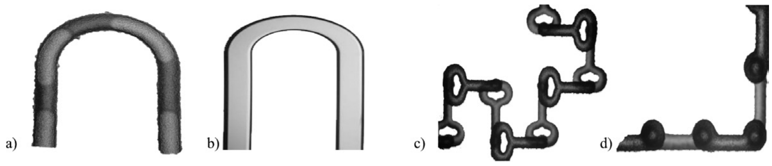

Fig. 3. Different types of microchannels: a) tubular microchannel with rough walls, b) tubular microreactor with smooth walls, c) teardrop micromixer and d) swirl micromixer

Table 1. Some advantages and disadvantages of microreactor

Advantages	*Fast mixing and mass transfer*	• diffusion limitations are minimised • no concentration gradient • mixing takes place by molecular diffusion
	High surface-to-volume ratio	• from 10,000 to 50,000 m^2/m^3 • efficient energy, mass and moment transfer
	Laminar flow	• better control of reaction conditions • favours modelling of reactions • provides high surface to volume ratio and interface area • eliminates back-mixing
	Small substrate volume	• significant cost savings • a lot of information about the process can be collected with small inlet volumes
	Environmentally friendly	• small substrate input and small product output • reduction in waste stream and total amount of waste • small energy consumption
	Safe reaction conditions	• small volumes, extensive heat transfer and variety of materials for production that can adapt to extreme reaction conditions (high pressure and temperature, explosive reaction etc.)
	Selectivity	• formation of purer products in shorter residence time
	Rapid reactions rate	• due to combination of previous mentioned advantages, reaction rates are faster on macro scale • higher space-time yield/productivities
	Numbering-up	• simple construction • uninterrupted continuous operation since a broken unit can be easily replaced without disturbing other units • chemistry performance of single unit is replicated on all other
	Easy to manipulate (Fig. 4)	• like LEGO system, microreactors can be easily assemble
Disadvantages	*Clogging* (Fig. 5)	• demands careful preparation of substrates (usually filtration before usage) • limits the usage of substrates
	Production price	• production of microreactor can significantly increase in cost depending on used material, production technique, complexity of the reactor • additional equipment (pumps, sensors etc.) also increase overall cost
	Handling the formation of solids	• if the product of the reaction is a solid, particles aggregate and cause blockage of the channel
	Significant effect of surface (microchannel wall)	• when working on a small scale surface roughness plays important role on the process
	Chemical adsorption on microchannel wall	• chemicals in solution adsorb on the channel walls thereby resulting in a loss of chemical concentration
	Industrial application	• still difficult to find general industrial application due to the production volumes

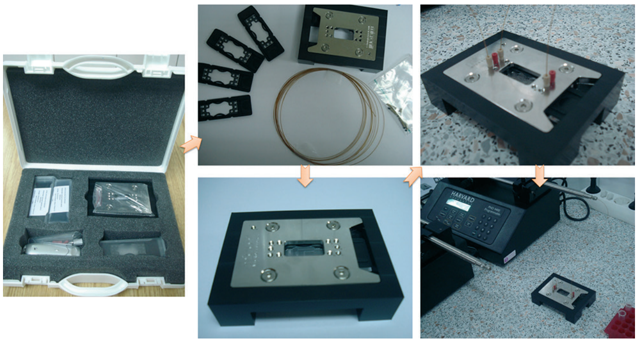

Fig. 4. Steps to assemble a microreactor (from a suitcase to a functional reactor system)

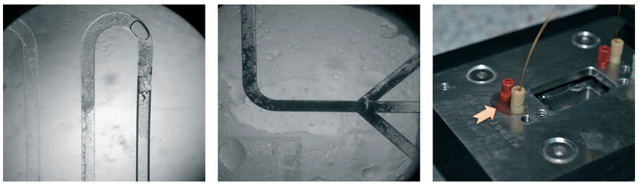

Fig. 5. Formation of clogging and leaking in a microreactor

2. Microreactor types

It is hard to make one uniform classification for different microreactor types. Usually the classification depends upon the aspect (microreactor physical characteristics, application or fabrication method etc.) from which the reactors are observed. According to the one classification [4], microreactors can be divided into two large groups: chip- type microreactors and microcapillary microreactors.

Chip-types are most often used. They offer better process control and easier integration in comparison to microcapillary microreactors. Microcapillary microreactors are usually produced from polymers like fluoropolymer microcapillary film (MCF) and they consist of larger number of parallel capillary channels with mean hydraulic diameters typically between 150 and 400 µm. Example of microcapillary microreactor are microcapillary flow discs used in chemical synthesis [5].

On the other hand, when scoping the literature or searching on the internet, terms like microreactor for chemical reactions, photocatalytic microreactors or enzymatic microreactors can be found. Consequently, most common classification is based on application of microreactors. Another classification of microreactors is based on production material so there are glass microreactors, stainless steel microreactors, polymer microreactors etc. Further classification is based on the shape of channel itself or upon different structures added in to the channel. In that case most common type of microreactors are microreactors with micromixers, zigzag microreactors, microreactors with nozzle injections etc.

Further classification can be based on microchannel properties, type of flow, number of phases etc. Some of them are listed in Table 2.

Table 2. Different microreactor types based on specific characteristics

Characteristics:	**Microreactor types:**
Flow type	• laminar flow microreactors • segmented flow microreactors
Design complexity	• basic (i.e. single tubular microreactor) microreactors • complex (i.e. μ- Total Analysis System) microreactors
Channel wall surface roughness	• smooth (relative roughness around 1%) microreactors • rough (relative roughness around 10%) microreactors
Shape of the inlet	• T microreactors • Y microreactors • ψ microreactors
Number of reaction phases	• single-phase microreactors • multi-phase microreactors
Durability	• disposable microreactors • reusable microreactors
Phase type	• liquid-phase microreactors • gas-phase microreactors
Application	• microreactors for (bio)chemical synthesis • microreactors for polymer synthesis • microreactors for process analysis • microreactors for material analysis
Fabrication material	• glass microreactors • ceramics microreactors • silicon microreactors • polymer microreactors • steel microreactors • perfluoroalkoxy (PFA) microreactors
Product type	• T, Y or ψ microreactors • falling fil microreactors
Mixing	• round bottom flask microreactor • jacketed microreactor • asia microreactor

3. Application of microreactors – bridging the gap between laboratory and industry

For many years now microreactor technology has been reserved mainly for laboratory research. Main focus in research was to collect as much as possible information about: (*i*) the transferring the process from batch regime to continuously operating regime, (*ii*) process optimization, (*iii*) production, (*iv*) kinetic measurements, (*v*) separation, (*vi*) discovering new production routes, etc. The biggest progress was made in chemistry especially in organic or polymer synthesis of a variety of products [6,7]. During the organic synthesis main challenges are usage of toxic chemicals and extreme conditions like high pressure and temperature. Microreactors present advantage by carrying such reactions in much safer environment [8]. In polymer synthesis it was reported that narrow particle size and molecular weight distribution was achieved in microreactor due to good efficient mass transfer and good heat removal [9]. Photochemical application of microreactors was also studied [10]. By combining the benefits of micro-scale with continuous-flow mode, microstructured reactors enable, when compared to conventional photochemical equipment, higher conversions and selectivities while reducing irradiation time. Additional benefit is more efficient light penetration, minimization of side reactions, easy control or irradiation time and safer conditions. Synthesis of nanoparticles in microreactors was demonstrated as potential alternative for large scale production of nanoparticles. Most progress was made on synthesis of inorganic and metal nanoparticles [11].

Of all the fields, biochemical and pharmaceutical application of microreactors is the last explored. Enzymatic microreactors for biochemical and pharmaceutical application can be roughly divided in to two groups, one includes biosynthesis and biochemical processes and second, screening, protein folding, enzyme kinetic estimation and analytical assays [3]. Enzymes as key element can be used in immobilized or in suspended form. Different approaches can be applied to immobilize enzyme in to microreactor. Most common are covalent immobilization, adsorption and co-polymerization [4]. The main problem in this field is that many microreactors that are well established for chemical productions can not be used for biochemical reactions. The first problem is residence time distribution since enzymatic reactions are usually slower then chemical. This leads to development of new microreactors that will satisfy demand for longer residence times. The second problem is catalyst lifetime because in comparison to chemical catalyst, enzymatic catalysts have shorter lifetime. As a solution, immobilization and development of new reactor systems that will enhance enzyme stability and activity are proposed. The third problem is cascade catalysis where one has to adapt different reaction conditions for different enzymes. As a solution, compartmentalization of microreactors is proposed [4].

Besides laboratory research, lately the interest is slowly shifting towards development of modular systems that should be the next step that is necessary if microreactors will be implemented in to the industry. Main idea is that these modular systems include all production steps – from substrate preparation to clean product ant the end of the process. Some systems presented by Corning and Chemtrix B.V. are already present on the market but there are still several disadvantages to overcome like numbering up, production costs, flow stability etc.

4. Microreactor technology in Croatia

As mentioned, microreactor technology is known and implemented in science for more than 40 years. The first reaction in the microchannels done by Croatian scientists was performed in the laboratory of Faculty of Chemistry and Chemical Technology, University of Ljubljana in Microprocess Engineering Research Group by prof. Tišma [12]. Laccase-catalyzed L-DOPA oxidation in an oxygen-saturated water solution was studied in a Y-shaped microreactor at different residence times.

In Croatia, a decade has passed since the first chips were assembled and tested for different processes. The first one was bought by the Faculty of Chemical Engineering and Technology, University of Zagreb and up to today they were applied mostly in biotransformation processes by group of Prof. Zelić. Main research focus of Prof. Zelić group was related to the production of valuable chemicals such as hexanol [13-15] and hexanoic acid [16], polyphenol oxidation [17,18] and coenzyme regeneration [19-22]. Besides that, application of microseparators for polyphenol extraction in two-phase aqueous systems was analysed [23,24]. Following the world trends, they were uses in the synthesis of different chemical compounds [25] and in photochemical processes. Applications of microreactor for biodiesel production and purification [26,27] as well as expanding the research on photochemical application of microreactors for production of pharmaceutical chemicals were set as future challenges in the same group by dr. Šalić.

The research group of Prof. Vrsaljko from the same Faculty is focused on utilising the 3D-printing technology for microreactor production from different polymers [28]. The combination of microreactors and 3D-printing reactors is also a worldwide growing terrain. Production of cheap but highly efficient reactors is something worth of the attention. Manufacturing of microreactors by 3D-printing technology is performed also in group of Prof. Šercer at Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb.

Two research groups from the Faculty of Food Technology and Biotechnology, University of Zagreb are also implementing microreactor technology in different fields. While group of Prof. Jurinjak Tušek is oriented towards mathematical modelling [29] of microreactor processes, the group of prof. Rezić is focused on dye decolourization processes and microreactor production by PolyJet Matrix Technology.

Recently, the research group from the Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, set-up a microreactor system and started with the research in the field of biotransformations of phenolic compounds originated from food industry waste.

5. Future perspective of microreactor technology

Worldwide, there are several key players when it comes to microreactor technology: Soken Chemical & Engineering Co., Ltd., Bronkhorst (UK) Ltd., Chemtrix B.V., Little Things Factory GmbH, Ehrfeld Mikrotechnik BTS GmbH, Micronit Micro Technologies B.V., AM Technology Co., Ltd., Corning Inc. and Vapourtec Ltd. They usually set the trends and these days; they are holding majority of microreactor technology market. There are many different predictions about the future trends of microreactors worldwide. Some estimation says that global microreactor technology market will reach 105 million USD by the end of 2025, and some even triple the mentioned number [30-32]. Although the numbers vary depending on initial parameters, all market researchers predict significant growth in the next five-years period. They estimate that the main focus of technology itself will be in expansion in regions with significant growth potential, such as India and China. Also, reduction of labour costs is expected and connected with high level of plants automatization. The predictions are that the switching from batch to continuous processes, will lead to the increase of labour costs between 10-20%, while the investments will decrease between 5 – 15%. Focus will also be on high speed development and high-performance products (i.e. development of new materials) together with process intensification (performance, stability etc.). It is believed that during process intensification, the yield should grow from 0 – 40% and energy should decrease from 5 – 15% and by shortening the time-to-market aspect development time should be reduced between 10-30%.

As for research area, three areas are marked as spotlights in the next time period: drug development processes, chemical production and biodiesel synthesis.

Chemical production in microreactors is present from the early days of microreactor technology, while drug development processes and biodiesel production drown the attention later on.

Drug development process is still considered as one of the most expensive processes. Because of that pharmaceutical companies are trying to find new development routs that will decrease time-to-market aspect, that will enable new process formation and increase production throughput. Microreactors are considered as good and efficient tools that could meet all those demands [33].

As for biodiesel production, biodiesel as an alternative fuel with low environmental impact become interesting with increasing concerns of global warming. Most common method of biodiesel production is transesterification. Application of microreactors in the production will allow the reaction to be carried out faster and higher gains will be achieved in reasonable period of time [26,34].

At the end, as mentioned before, researches, by developing robust modular systems, are working on how to bridge the distance between laboratory and the industry. It is believed that application of microreactors in the industry will have several advantages in comparison to traditional macro reactors as follows:

1. *technical*, meaning better process control, efficient heat transfer and better performance at extreme conditions
2. *ecological*, meaning safer production of chemical and pharmaceutical products, smaller usage of chemicals and reduction of waste

3. *economical*, meaning reduction of costs by implementing numbering-up instead of scale-up and″integration of different production steps.

Going towards industry is the final challenge that microreactor technology will face in the future.

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Production of green note component on a microscale – From idea to integrated systems

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Abstract

In comparison with macro level, batch production processes development of continuous, micro level production processes presents a certain challenge since a lot of new physical phenomena emerge. Different heat and mass transport phenomena, different diffusion time, enhanced surface roughness impact, different flow profiles etc. are just some of the challenges that have to be overcome in order to develop a functional production system on a microscale. As a case study for this statement production of hexanal has been investigated. Different experimental approaches for production in a microreactor were necessary in order to transfer already established microscale batch production. While "one pot" principle was working perfectly for batch production, it was necessary to develop an integrated system with several connected microchips for successful and effective hexanal production on a microscale. In this paper, basic steps that led from idea to integrated system of hexanal production on a microscale are demonstrated.

Keywords: *hexanol oxidation, hexanal, microreactor, alcohol dehydrogenase, coenzyme regeneration, immobilization*

1. Introduction

Hexanal is part of the group of natural volatile chemicals, the so-called "*green notes*". "*Green notes*" (aldehydes and alcohols) are high-value molecules widely used in the aroma industry to impart the green character associated with freshness. Hexanal has a pleasant grassy odor and its organoleptic fresh note is what makes it so interesting for consumers [1].

Nowadays, several methods are applied for hexanal production based on fermentation, extraction from plants, and enzyme-catalyzed reactions [2]. Main problem of these traditional methods is small amount of produced hexanal. Additionally, fermentation processes and extraction from plants result with formation of large amounts of unwanted by-products and a lot of waste. Main problem of the enzyme catalyzed synthesis is low yield. The general opinion is that the demand for hexanal would increase if it could be produced in a more economical way. In addition, although the yield is low, the use of enzymes for catalysis compared to classical chemical catalysts is highly desired in food industrial processes, because the resultant products would be classified as "natural" by food regulatory agencies, a feature that increases their public acceptance as ingredients for food [3].

Following that idea Vrsalović-Presečki [4] demonstrated that hexanal can be produced by oxidation of hexanol using NAD(H) dependent alcohol dehydrogenase (ADH) from baker's yeast as catalyst in a batch reactor ($V = 10$ mL). Using equimolar concentration of hexanal and coenzyme 5.3% conversion was obtained during 3 min. Results indicated that coenzyme regeneration is necessary, not only to reduce process costs, but also to shift the production in the direction of products. When enzyme regeneration system based on acetaldehyde reduction was introduced into the process (Fig. 1) conversion in a batch reactor increased to 11% (t = 25 min). Although the results demonstrated that it was possible to produce hexanal by this approach, conversion was still too low.

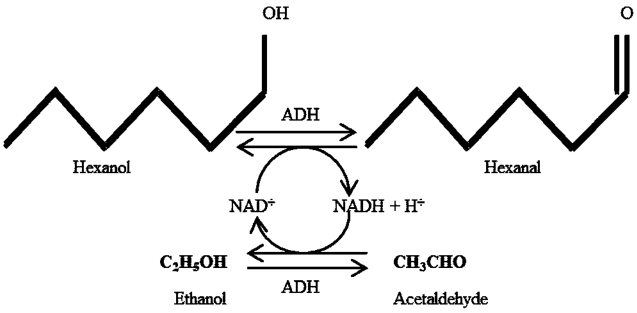

Fig. 1. Reaction scheme of ADH-catalysed hexanal oxidation with NADH coenzyme regeneration based on acetaldehyde reduction

By combining enzymatic biocatalysis and microreactor technology, a new way for hexanal production was proposed. Microreactor technology is a new interdisciplinary technology. Benefits of this new technology pose a vital influence on chemical industry, biotechnology, the pharmaceutical industry and medicine. A high surface to volume ratio, faster diffusion dominated transport, enhanced heat transfer and thus reduced energy demands, good process control, high throughput, usage of minimal (microlitres) of reagent volumes, etc., are some of a microreactor advantages that are usually stressed [5].

2. State of the art

Within this paper application of microreactor technology for hexanal production is presented. Overall research was divided in to three phases. *First phase* was focused on hexanal production on a microscale, *second* on coenzyme regeneration and *third* on the development of integrated system for parallel hexanal production and coenzyme regeneration. The idea behind integrated system (Fig. 2) was to introduce hexanol dissolved in hexane as one process phase and enzyme and coenzyme dissolved in buffer as second phase. After introducing the phases, it was necessary to accomplish stable and parallel fluid flow to separate the phases at the end of the first microreactor. At the outlet of the first reactor, an aqueous phase containing enzyme and coenzyme (NADH and NAD$^+$) dissolved in buffer would be directed in to the second microreactor in order to regenerate the coenzyme. Acetaldehyde dissolved in buffer would be used for coenzyme regeneration.

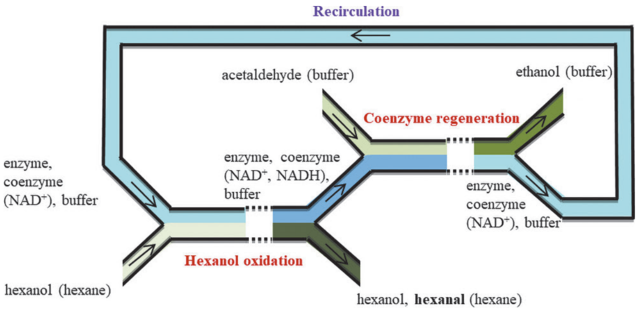

Fig. 2. Process scheme for hexanol oxidation with coenzyme regeneration and recirculation

The outlet of the second microreactor containing regenerated coenzyme and enzyme would then be reused again (recirculation) and fed as the second stream in to the first microreactor. Main goal was to increase the conversion and yield and reduce production time to make the process sustainable.

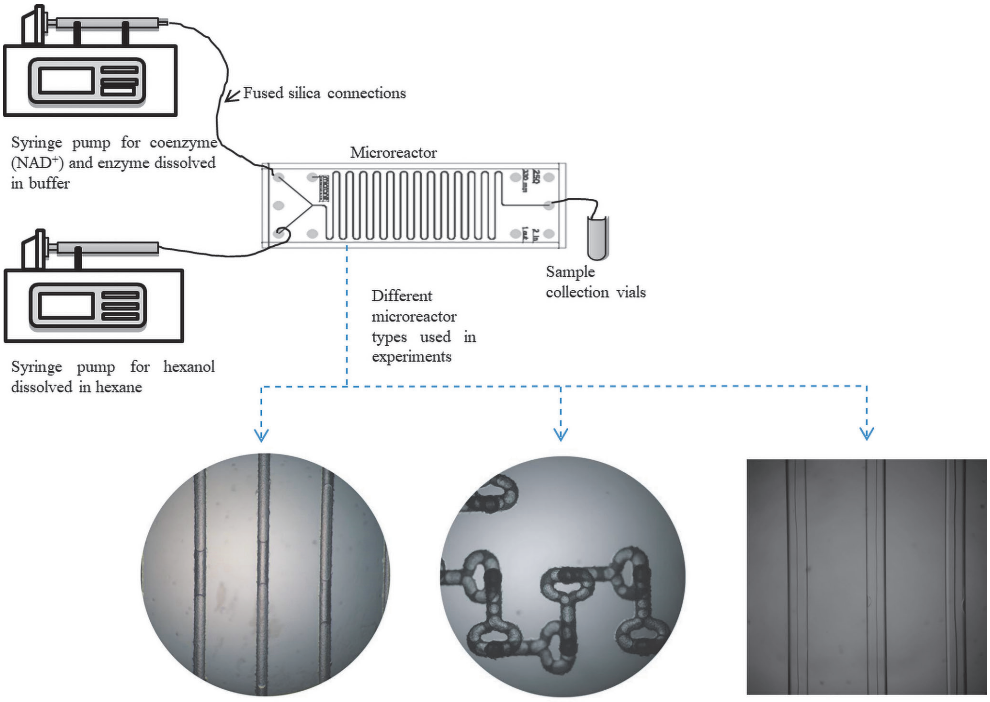

Fig. 3. Process scheme and reactor types used for hexanal production in a microreactor

3. Results

3.1. Hexanal production in a microreactor

As mentioned, first phase was focused on hexanal production on a microscale. Same reaction mechanism as the one described by Vrsalović-Presečki [4] was chosen, meaning hexanol oxidation was performed by using NAD(H) dependent alcohol dehydrogenase (ADH) from baker's yeast. Besides purified enzyme, permeabilized baker's yeast cells were also used as a potential source of ADH.

Permeabilized baker's yeast cells were used as inexpensive and easy to obtain. Additionally, in order to overcome some disadvantages of suspended biocatalyst usage, like decrease of stability during storage, complicated catalyst-product separation, harder operation and impossible reuse, biocatalyst immobilization was investigated for both types of biocatalysts, purified enzyme and permeabilized baker's yeast cells, respectively.

Different types of microreactors:

(*i*) tubular microreactors with rough walls with internal volume of 6 µL and 13 µL,
(*ii*) tubular microreactor with smooth walls and
(*iii*) microreactor equipped with micromixers,

different inlet concentrations of substrate and biocatalyst as well as different flow rate ratios of the organic and aqueous phase were investigated in order to propose the best reactor type and best process conditions for hexanal production.

Comparison of results for different process conditions are shown in Table 1. Simplified process scheme and different microreactor types are presented in Fig. 3.

Obtained results, for every investigated microreactor system, indicated that microreactors could be a good choice for hexanal production in comparison to traditional (batch) production process where, as mentioned, conversion of 5.4% was achieved in 3 min. Best results in microreactors (Table 1) were obtained by using suspended enzyme in microreactor with rough walls (experiment 13) and equimolar concentration of substrates and in microreactor with smooth walls with concentration of coenzyme 10 fold lower than the concentration of the hexanol (experiment 16).

As already mentioned, in order to develop integrated system it was necessary to establish stable and parallel fluid flow that will allow phases separation at the end of the microreactor.

Using the microscope and staining the aqueous phase with blue dye, formation of the flow patterns was observed. It was noticed that in the microchannel with rough walls formation of segmented flow is more characteristic while in microchannel with smooth walls a stable and parallel flow is developed (Fig. 4).

Table 1. Comparison of the highest conversion of hexanal under different reaction conditions

Experiment	**Experimental conditions**										**Results**		**Reference**
	catalyst form	**inlet concentration**			**flow**			**microreactor**			τ (s)	X (%)	
					ratio								
		$c_{i,\text{hexanol}}$ (mmol L^{-1})	c_{i,NAD^+} (mmol L^{-1})	$\gamma_{i,\text{ADH}}$ (g L^{-1})	aqueous	organic	profile	type	V (μL)	surface roughness			
1-4	suspended enzyme	5.5	0.55	0.92	1	1	segmented	tubular	6	rough	7.2	7.8	[6]
		5.5	0.55	0.092	1	1					72	11.8	
		5.5	1.1	0.092	1	1					4.8	14.3	
		5.5	11	0.92	1	1					4.8	9.1	
5	suspended enzyme	5.5	0.55	0.092	1	1	segmented	tubular	13	rough	78	11.3	
6	suspended enzyme	5.5	0.55	0.092	1	1	segmented	micromixers	2	rough	12	10.9	
7-9	suspended enzyme	5.5	0.55	0.092	1	3	segmented	tubular	6	rough	90	11.3	
					1	5					60	9.7	
					1	10					81.8	11.5	
10-12	suspended enzyme	5.5	0.55	0.092	3	1	segmented	tubular	6	rough	90	11.4	
					5	1					60	10.4	
					10	1					81.8	7.9	
13	suspended enzyme	4.4	4.4	0.092	1	1	segmented	tubular	6	rough	72	80	[7]
14	immobilized cells	5.5	–	–	–		–	tubular	6	rough	20	8	[8]
15	suspended cells	5.5	–	–	–		–	tubular	6	rough	2	24	
16	suspended enzyme	5.5	0.55	0.092	1	1	parallel	tubular	6	smooth	20	53	[2]
17	suspended enzyme	5.5	0.55	0.092	3	1	parallel	tubular	6	smooth	10	19	

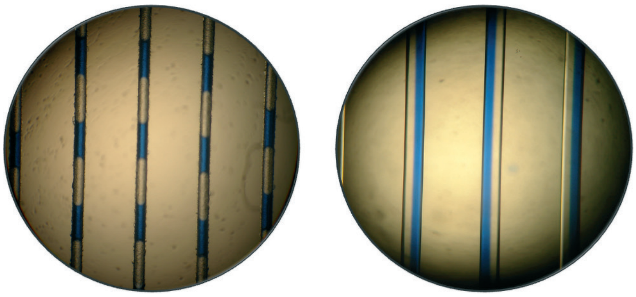

Fig. 4. Microscopic observation of the flow pattern formation in microreactor with rough and smooth channel walls (blue – aqueous phase, colorless – organic phase)

Another effect was noticed when both phases enter the reactor with same velocity. In that case less viscous hexane occupies a much smaller part of the channel and the interphase area between two phases is stable but not formed in the middle of reactor (Fig. 5). This presented a problem for phase separation at the exit of the microreactor. Phase separation was necessary for enzyme regeneration and recirculation as well as for product separation.

In order to resolve this problem, hexane flow velocity was elevated and the new flow ratio of aqueous:organic phase of 1:3 was proposed. Unfortunately, this lead to decrease of conversion but obtained result was still higher than those obtained in batch reactor (Table 1, experiment 17) and phase separation was now possible.

3.2. Coenzyme regeneration in a microreactor

Since the price of coenzyme, which is essential for ADH functionality, coenzyme regeneration process in a microreactor was second phase of process development. Coenzyme must be added in reaction in a stoichiometric amount and may not be replaced by more economical synthetic products. Enzyme ADH used as the biocatalyst in the hexanal production process was also used for regeneration of coenzyme. Other substrate, acetaldehyde was used as a substrate for coenzyme regeneration because of its low price and the high specificity of ADH towards it. Disadvantages of selected reaction system were the possibility of the enzyme deactivation both by substrate, acetaldehyde, and the product, ethanol, instability (possibility of self-condensation in the solution) and volatility of acetaldehyde [9].

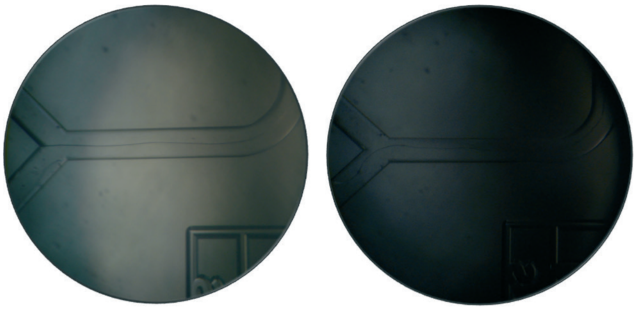

Fig. 5. Microscopic observation of the flow patterns at the exit of the microchannel for the flow ratio of aqueous and organic phase 1:3 and 1:1

Table 2. Comparison of the different systems used for NADH regeneration

Experiment	Experimental conditions							Results		Reference
	catalyst form	inlet concentration			microreactor					
		$c_{i,\text{acetaldehyd}}$ (mmol L^{-1})	$c_{i,\text{NADH}}$ (mmol L^{-1})	$\gamma_{i,\text{ADH}}$ (g L^{-1})	type		V (μL)	τ (s)	X (%)	
1	suspended enzyme	5.5	6.9	0.2	glass microreactor		6	2	80	[10]
2	suspended enzyme	44	6.9	0.2	glass microreactor		6	0.8	100	
3	suspended cells	5.5	5.5	0.1	glass microreactor		6	36	65.3	[11]
4	immobilized enzyme	5.5	5.5	–	glass microreactor		6	3.6	12	
5	immobilized cells	5.5	5.5	–	glass microreactor		6	7.5	3.6	
6	suspended cells	5.5	5.5	0.1	PTFE microreactor		273.15	47.1	86.67	
7	suspended enzyme	5.5	5.5	0.1	PTFE microreactor		273.15	47.1	94.35	
8	immobilized enzyme	5.5	5.5	–	PTFE microreactor		273.15	94.3	11.91	
9-11	enzyme loaded on magnetic nanoparticles	5.5	5.5	–	PTFE microreactor with	square magnet	273.15	10 (min)	100	[12,13]
		5.5	5.5	–		cylindrical magnet		6 (min)	96.4	
		5.5	5.5	–		electromagnet		180	100	

These problems could be solved by using continuously operated microchannel system at different flow rates that could provide short contact time between enzyme and the components with inhibition effect. Summary of the conditions and results are presented in Table 2.

Different forms of biocatalyst (suspended and immobilized enzyme and permeabilized baker's yeast cells) in different reactor types (*i*) glass microreactor with smooth walls, (*ii*) PTFE (polytetrafluoroethylene) and (*iii*) microreactor with and electromagnet or oscillating magnetic field that allowed better distribution of biocatalyst immobilized on nanoparticles were tested to find the best solution for coenzyme regeneration.

In order to generate magnetic field to utilize magnetic properties of magnetic nanoparticles as carrier for biocatalyst, a system for magnetic field generation and regulation was developed (Fig. 6). According to Derks et al. [14] in magnetic bead motion within a fluid, the magnetic and drag forces dominate the bead motion, since at the micrometer scale, effects of gravity and inertia become very small. Therefore, a bead will almost instantaneously accelerate to its terminal velocity at which the magnetic and drag force are exactly at balance with each other.

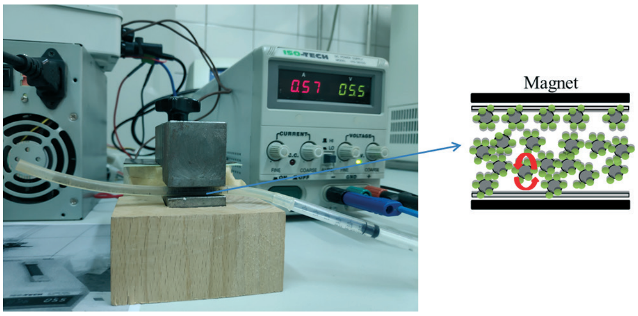

Fig. 6. Experimental set up used for magnetic field regulation developed at University of Zagreb, Faculty of Chemical Engineering and Technology

Using this concept, it was possible to actively move or restrain the particles across the channel. If the oscillating magnetic field is applied constantly, particles will move towards both the north and south poles. Particle oscillation is obtained by switching the poles on and off. When one pole is active, the particles are attracted to it. When the field is switched and the poles are switched, the beads will be attracted towards opposite direction.

When the results of all experiments were compared the best results (Table 2) were obtained by using suspended

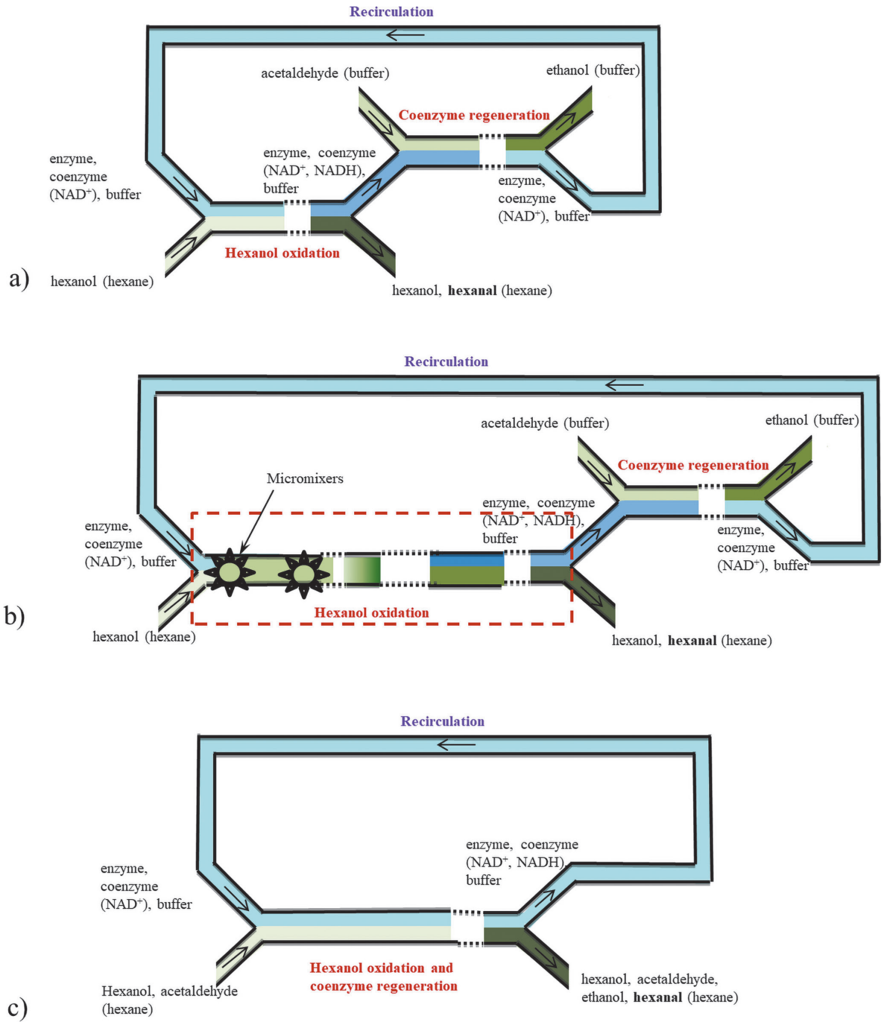

Fig. 7. Different process schemes for hexanal production in integrated systems

enzyme in a microchannel with smooth walls. A conversion of 100% was obtained for the residence time less than 0.8 s when acetaldehyde was in excess. Therefore, this type of the reactor and excess of acetaldehyde in reaction mixture were proposed as the best reaction system for further development of completely integrated system for hexanal production.

3.3. Production of hexanal with in situ product separation – integrated system

In order to develop the production of hexanal with *in situ* product separation all obtained results were analyzed and three different microreactor systems were proposed (Fig. 7).

In the *first system* two microreactors were connected into the series (Fig. 7a). First chip was used for hexanol oxidation and second for coenzyme regeneration. After regeneration, regenerated coenzyme was recirculated into the first microreactor where oxidation step was continuously performed. During the first 4 hours of continuous production, a maximal conversion of 19.5% was achieved. In that period, amount of produced hexanal didn't change but, prolonging the process time, a significant decrease in production was noticed. In 3rd day, process stopped since the activity of the enzyme decreased and only 1% of hexanal was measured at the entrance of microchannel. The biggest advantage of this system was that no additional enzyme and coenzyme were added in the process for all 3 days.

Based on the results presented in Table 1 (experiment 3), where high conversion [6] of hexanol to hexanal were achieved in the microreactor equipped with micromixers, a *second system* was developed (Fig. 7b). It was basically the upgrade of the first system where one microreactor for hexanol oxidation was replaced with two microreactors connected in to the series. First microreactor was microreactor equipped with micromixer and second one was tubular microreactor. On one of the previous research [15] it was noticed that when microreactor chip with micromixers and microreactor with smooth microchannel were connected in series it was possible to enhance mass transfer and separate flows at the exit of microreactor. This would allow regeneration in the third microreactor chip. Unfortunately, despite the literature and previously obtained results, it was not possible to achieve stable flow with the interphase positioned exactly in the middle of second microchannel proving that this developed system was not sustainable for development of integrated system for hexanol oxidation.

Finally*, third system* that was developed was downsizing of the first system (Fig. 7c). The main idea was to make the integrated process as simple as possible, so oxidation and regeneration were placed on the same chip. Maximal conversion obtained at the beginning of the experiment was 17.1% but enzyme activity decreased (deactivation by organic components) rapidly and the process stopped after 1.5 day.

4. Conclusion

Taking all the results into consideration, we believe that with some additional optimization, and further production cost projections, microreactors could serve as the next-generation production process not only for hexanal production but also for the fast and efficient production of different fine chemicals and pharmaceuticals as well as for production of large-scale products.

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Sustainable biodiesel production on micro-scale

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Abstract

Due to the fact that fossil fuel resources are decreasing significantly and that their usage has a negative impact on the environment, there is a constant search for alternative fuels. Biofuels, especially biodiesel, attract more and more attention since they are usually considered as environmentally friendly. However, conventional methods of biodiesel production include the application of large amounts of acids or bases together with large amounts of water needed for purification. Therefore, a question mark can be put on the term environmentally friendly. The most common industrial process of biodiesel production is transesterification of different oils with methanol in a batch reactor using acids or bases as catalysts. Alternative production paths are constantly being explored. One of the solutions to maximize production efficiency and minimize the production costs is the use of enzymes as catalysts in a continuous process. Advantages of continuous biodiesel production processes are being more and more explored whereby application of microreactors results with many advantages comparing to conventional, batch and macroscale processes. In this paper, a short overview of different microreactor types for biodiesel production has been presented.

Keywords: *microreactors, biodiesel, transesterification*

1. Introduction

Pursuant to the Biofuels for Transport Act (OG65/09, 145/10, 26/11 and 144/12), biodiesel, as one of the biofuels used for transport, is defined as a fatty acid methyl ester (FAME), produced from vegetable or animal oil, of diesel quality, to be used as biofuel. The main advantages of biodiesel compared to conventional fuels are increased biodegradability, increased lubricity, high flash point, non-toxicity, and reduced emissions of hydrocarbons, sulphur, carbon monoxide and particulate matter [1-4]. The main disadvantages of biodiesel production and application are especially high raw material prices (75% of the total costs of biodiesel production [5]), stability in storage and after exposure to atmospheric conditions, increased NOx emissions, low calorific value and poor low temperature properties [6-9]. There are a variety of methods used in biodiesel production (Fig. 1).

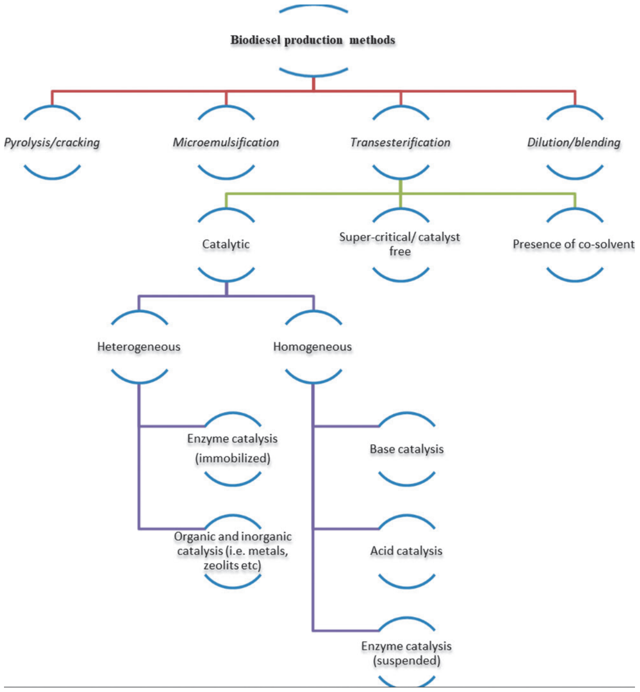


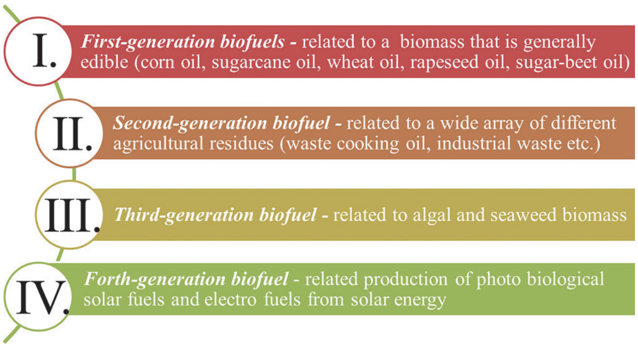
Fig. 1. Different methods for biodiesel production

Microemulsification [7] is based on blending animal and vegetable oils with solvents and microemulsions or surfactants, to form a microemulsion biodiesel fuel. *Pyrolysis* [10-14] is based on heating dried biomass without oxygen in a reactor at a temperature of about 500 °C with subsequent cooling. *Blending* is based on blending vegetable oils as biodiesel directly with conventional diesel fuels in a suitable ratio. Currently, *catalytic transesterification* is the most important method for the industrial production of biodiesel [15-19].

On the industrial scale, transesterification (or alcoholysis) is nowadays mostly performed in batch reactors. Different biomass and feedstock like edible vegetable oil, animal fats, waste edible oil remaining after roasting and non-edible oil [20] in combination with short-chain alcohols (methanol and/or ethanol) can be used as substrates for the biodiesel production.

Based on the source of biomass biofuels, in general, can be divided into four generations as shown in Fig. 2. Biodiesel is mostly produced by feedstock from the first and second generation.

Transesterification under moderate conditions is not possible without the catalyst. The selection of the catalyst depends on the feedstock used. Homogeneous, heterogeneous and enzymatic catalysts can be used in the biodiesel production. Homogeneous catalysts in the alkali-based process (with NaOH and/or KOH) or in the acid-based process (with sulphuric acid) are commonly used in industrial production. The disadvantages of the alkali-based process include the undesirable saponification side reactions that lead to the formation of emulsions and lower ester yield. Acid-catalyzed transesterification requires higher amounts of the catalyst but function better at higher alcohol-to-oil ratios. Regarding downstream, more process steps are required in acid-catalyzed production, which is more energy and economically demanding. Moreover, acids can cause equipment damage. Generally, huge amounts of wastewater are generated during chemically-catalyzed biodiesel production, which is environmentally unfriendly [21].



First-generation biofuels - related to a biomass that is generally edible (corn oil, sugarcane oil, wheat oil, rapeseed oil, sugar-beet oil)

Second-generation biofuel - related to a wide array of different agricultural residues (waste cooking oil, industrial waste etc.)

Third-generation biofuel - related to algal and seaweed biomass

Forth-generation biofuel - related production of photo biological solar fuels and electro fuels from solar energy

Fig. 2. Generations of biofuels production

Due to high costs and low reaction rates in these processes, the formation of by-products in parallel, side reactions, necessity to neutralize the catalyst and high energy consumption, possibilities of applying some other catalysts are being explored. Significant process improvements are predicted by the implementation of transesterification using enzyme as a catalyst. The application of enzymes in the transesterification process, comparing to acids and bases as catalysts, has several advantages. Besides the fact that enzymatic processes are performed under mild conditions, enzymatic biodiesel production leads to the production of food-grade glycerol without soap formation.

Some of disadvantages and limitations in biodiesel production by the method of transesterification together with the suggested solutions are listed in Table 1.

In order to overcome the mentioned limitations and to improve the process, advantages of continuous production of biodiesel in microreactors are being widely explored. Intensive mass and heat transfer in microreactors, which is a result of the high surface-to-volume ratio of the reactor, leads to a higher reaction rate and consequently to considerable savings in energy and raw material consumption. The high surface-to-volume ratio is a result of the reactor's structure, regarding the fact that a microreactor is a build-up of microchannels, whose dimensions are 10 µm – 500 µm, whereby microchannels are made by cutting on a solid plate. Flow in microchannels is mostly laminar due to their small diameter which consequently allows more precise regulation of the process. In addition, their reduced dimensions significantly reduce implementation and operational costs [25]. Xie et al. [26] outlined the main features of biodiesel production in microreactors compared to the conventional reactor systems such as the smaller volume of the reactor, reduction in the total size of the plant, higher surface area-to-volume ratio, higher productivity, higher efficiency of mixing and heat transfer, savings in capital costs and production costs and less energy consumption.

The enzymatic process for the production of biodiesel is simpler and more environmentally friendly than the chemical one [27-29]. The basic advantages of enzymatic processes in relation to the chemical procedures are mild reaction conditions (temperature 30 to 60 °C), separation of a large part of glycerol without further purification and without creating chemical waste, and the ability of lipase to catalyse the process of esterification of free fatty acids present in oils so that the requirements for purity of the starting materials are significantly milder. Using enzymes as catalysts in the transesterification reaction does not generate by-products as soaps, which significantly facilitates additional purification procedures and results in quality biodiesel. By using biocata-

Table 1. Disadvantages and limitations of biodiesel production by transesterification and possible solutions

Disadvantages and limitations	Solution
Reaction rate can be limited by a mass transfer between immiscible alcohol and oil phases	Application of microreactors
The transesterification reaction is a reverse reaction	Application of continuous removal of the product or increasing the alcohol to oil molar ratio
The majority of commercially used processes are based on biodiesel production in batch reactors [22]	Exploiting the advantages of continuous production processes by implementation of microreactors
The price of biodiesel is 1.5- to 3-fold higher than the price of diesel derived from fossil fuels [1,23]	Making the process more sustainable. Search for a) novel catalysts (obtained from waste materials, such as e.g. flying ash) and biocatalysts; c) lowering the cost of enzyme production by genetic engineering or search for novel microorganisms producers, d) application of immobilized enzymes using novel materials for immobilization; e) use of feedstock such as non-edible oil, waste frying oil, and oil with high FFA content
Long reaction time (the catalytic processes with acids and alkalis can take from 2 to 24 h) [24]	Application of supercritical conditions (temperatures up to 300 °C and pressures higher than 40 MPa) or addition of solvents that shortens the process of biodiesel production to just a few minutes

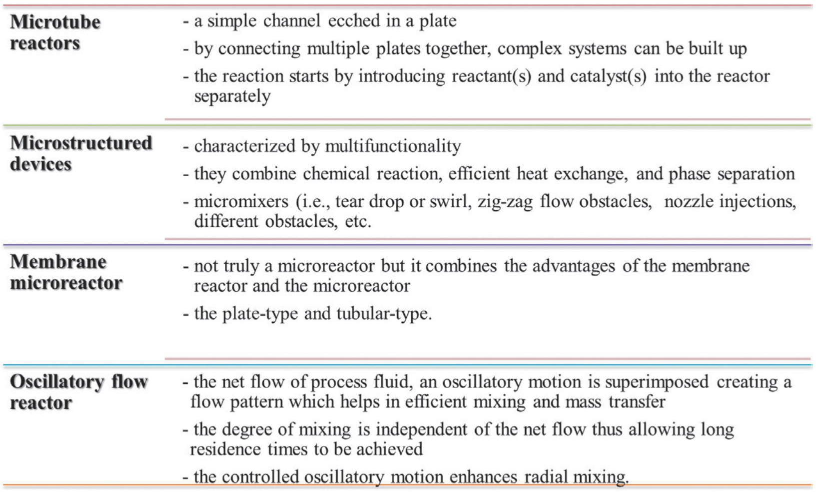
lysts, there is no need to rinse product in the final stage of biodiesel synthesis and purification, which reduces the amount of wastewater that poses a serious environmental problem in conventional biodiesel production.

Despite numerous advantages, enzymatic methods are still not competitive with chemical processes. The main obstacle for the introduction of enzymatic production processes is the high cost of enzyme and its reduced activity and stability in the presence of polar alcohol such as methanol and ethanol. Some of these deficiencies could be resolved by applying microreactors, which are still underexplored in terms of the enzyme-catalysed process of biodiesel.

2. Biodiesel production on a microscale

For some time now microreactors have been tested for biodiesel production [26,30,31]. Thanks to their flexibility in design different varieties of microdevices emerged for biodiesel synthesis. Their main goal was to enhance mass transfer to carry out the reaction in short residence time. Microreactors are known to considerably increase the dispersion of two phases as needed for the biodiesel reactants (alcohol and oil). This provides a much higher interface area that by the elimination of mass-transfer hindrance has shown to lead to shorter reaction time [32]. The capillary microreactor was the first one reported for biodiesel production and those that followed had more complex and advance design [33]. The most used microstructured devices for biodiesel production are listed in Table 2.

Table 2. Microstructured devices for biodiesel production



Microtube reactors	- a simple channel ecched in a plate - by connecting multiple plates together, complex systems can be built up - the reaction starts by introducing reactant(s) and catalyst(s) into the reactor separately
Microstructured devices	- characterized by multifunctionality - they combine chemical reaction, efficient heat exchange, and phase separation - micromixers (i.e., tear drop or swirl, zig-zag flow obstacles, nozzle injections, different obstacles, etc.
Membrane microreactor	- not truly a microreactor but it combines the advantages of the membrane reactor and the microreactor - the plate-type and tubular-type.
Oscillatory flow reactor	- the net flow of process fluid, an oscillatory motion is superimposed creating a flow pattern which helps in efficient mixing and mass transfer - the degree of mixing is independent of the net flow thus allowing long residence times to be achieved - the controlled oscillatory motion enhances radial mixing.

From the catalyst point of view, when comparing the number of transesterification reactions catalysed by chemical or by the enzymatic catalyst, up to now, most of the processes were oriented towards the application of chemical catalysts. Despite the advantages of enzymatic methods, as mentioned before, they are still not competitive to chemical processes [33]. Lipases have attracted the most attention for biodiesel production since they can catalyse hydrolysis, esterification and transesterification. Besides that, lipases are among the least expensive enzymes. Mostly used lipases for biodiesel production are from genus *Candida* and *Thermomyces lanuginosus* [34].

Overview of some transesterification processes performed on a microscale is presented in Table 3.

There are several different parameters that affect transesterification process on a microscale. They are presented in Fig. 3.

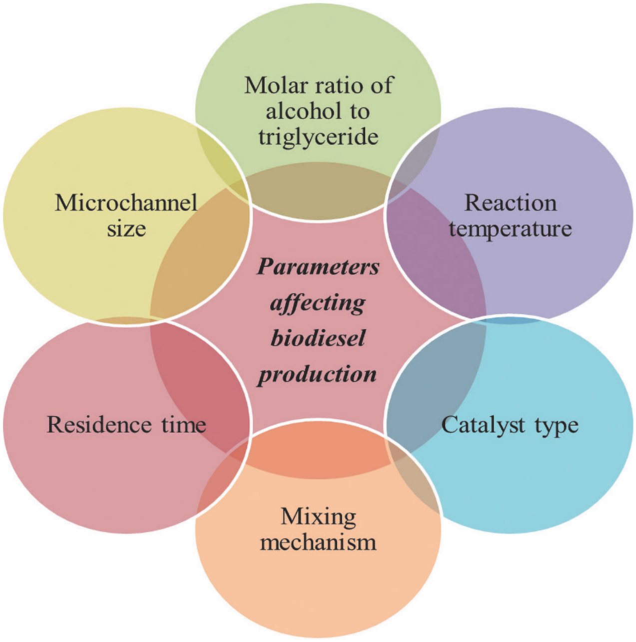


Fig. 3. Parameters affecting biodiesel production in microreactors

One of the most important parameters that has a significant effect on biodiesel productivity is the *molar ratio of alcohol to triglyceride*. Since transesterification is an equilibrium reaction, the excess of alcohol is needed to shift the reaction towards the formation of esters [30]. On the other hand, excess of alcohols makes the recovery of glycerol difficult. A second important parameter for biodiesel production on the microscale is a *microchannel size*, since reducing the channel size higher yield of es-

Table 3. Production of biodiesel by transesterification on a microscale

Microreactor type	Substrate	Catalyst	Residence time	Yield (%)	Reference
Microtube	Soybean/methanol	NaOH	10.36 min	91	[35]
Microtube	Waste cooking oil/methanol + *n*-hexane/tetrahydrofuran at 62 °C	Kettle limescale	10 min	97.03	[36]
Microtube	Oleic acid/methanol and carbon dioxide as solvent at 60 – 120 °C and 10 mPa	H2SO4	1 min	90	[37]
Microtube	Sunflower oil/methanol at 75 °C	NaOH	1 min	99.9	[38]
Microtube	Soybean/methanol	KOH	3 min	99	[39]
Microtube	Sunflower oil/methanol	Lipase	2 h	95	[40]
Microchannel with circular obstruction	Sunflower oil/methanol at 50 °C	KOH	12 s	99.99	[41]
Four active micromixer and magnetic field	Soybean/methanol	KOH	8 s	98.1	[42]
Microreactor with micromixers	Soybean/ethanol	KOH	4.9 s	96.1	[43]
Membrane microreactor	Triolein/methanol	Lipase	19 min	80	[44]
Oscillatory flow reactor	Sunflower oil/methanol at 50 °C	NaOH	30 min	98	[45]
Jacketed stainless steel tubes	Jatropha oil/methanol	NaOH	5 min	94	[46]
Zig zag channel shape	Soybean oil/methanol	KOH	28 s	99.5	[47]

ters can be achieved. The *reaction temperature* is especially important for reactions catalysed by enzymes since most of them have specific temperature optimum. For lipase from *Thermomyces lanuginosus* temperature optimum is around 40 °C. Reactions that are performed at higher temperatures are faster than those performed at room temperature (Table 3). *Mixing mechanism* has a significant impact on mass transfer which is especially important when immiscible phases (like oil and alcohol) are part of the reaction mixture. In order to enhance mixing different structures can be incorporated as part of the microchannel: micromixers (i.e. teardrop or swirl), zigzag flow obstacles, nozzle injections, etc.

Although the transesterification can be performed in the absence of biocatalyst (in that case the high temperature and pressure are required), the addition of *catalyst* allows moderate reaction conditions. Catalysts can be divided into homogenous and heterogeneous. Base and acid catalysts belong to the homogeneous group and are used commercially while heterogeneous (i.e. enzyme lipase) still need to undergo significant research.

3. Biodiesel purification on a microscale

Once biodiesel is produced, it must be purified. The purity of biodiesel according to the HRN EN 14214, must be ≥ 96.5%. Classical purification processes that are used in industry include removal of alcohol excess, glycerol, soap, unreacted triglycerides and catalyst. The first step is usually sedimentation, based on the difference in density of biodiesel and glycerol (Fig. 4a). The upper layer (biodiesel) is then removed and due to the solubility of glycerol in the aqueous phase, biodiesel at the end contains small amounts on impurity [48]. Wet washing (purification of biodiesel by water) is currently the most commonly used purification method for industrial biodiesel production. The biggest disadvantage of this approach is the generation of large amounts of waste-water [49]. In addition, it results in a loss of biodiesel during the washing phase. An alternative method would be dry washing that includes different absorbents, adsorbents, solvents and ion exchangers based on a variety of resins or applications of deep eutectic solvents [40].

When all the mentioned processes are performed on a macroscale and as batch processes (Fig. 4a) a lot of additional equipment is necessary to fulfill every step. The biggest advantage of a microreactor is the possibility to integrate all of the mentioned steps on a few chips (Fig. 4b). As presented by Šalić et al. [39], an integrated system, with enzymatic production of biodiesel on the first chip and purification on the second, can easily be assembled and successfully applied.

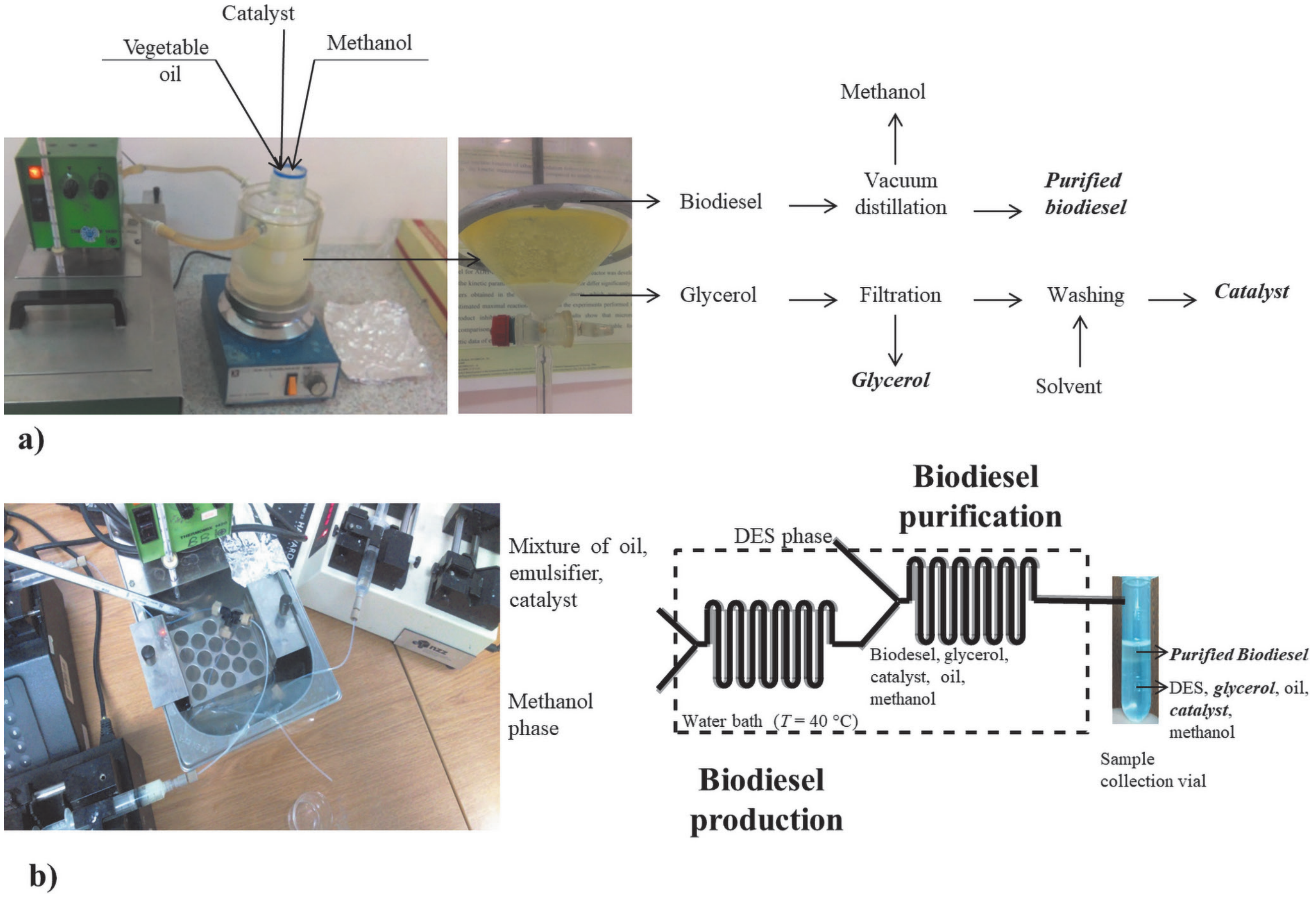


Fig. 4. Comparison of different steps in enzymatic biodiesel production and purification in a) a batch reactor and b) in a microsystem

Development of the integrated systems, which would include production and purification of biodiesel, glycerol purification and catalyst recovery, is one of the goals of future research in the area of biodiesel production on a microscale.

4. Conclusion

The application of microreactors for biodiesel production is promising and prospective technology. Although this technology still needs some improvements especially in the area of reactor production costs and biodiesel production capacity, obtained results (the high FAME yield, the high productivity, the short residence time etc) are in favour of microreactors. Nowadays we are in an era where the application of the small microreactor based portable factories that could be used in households or restaurants for biodiesel production from waste cooking old is not far away. Their development is for sure less complicated and expensive than classical scale-up of the batch biodiesel production process so progress in this area is expected in the near future.

Acknowledgments

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Mathematical models for description of hydrodynamics, mass and heat transfer in microreactor systems

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Abstract

Over the last decades the increased use of microreactors in all types of industries is observed. This phenomenon is based on the advantages that the microreactor systems can achieve in term of better process control, more efficient heat transfer and handling of hazardous chemicals. During this time mathematical models that describe flow inside microchannels have emerged ranging from Navier-Stokes equations to simplified mathematical models for laminar and segmented flows. The aim of this review is to present current studies in term of mathematical models that are nowadays commonly used for the description of hydrodynamics, mass and heat transfer in microreactor systems.

Keywords: *microreactors, mathematical models, hydrodynamics, mass and heat transfer*

1. Introduction

When talking about mathematical modelling in microreactors nowadays most people almost instantly think about computational fluid dynamics (CFD) and the results that are presented as a colourful pictures or moving simulations. CFD, as Zawawi et al. [1] explained, provides numerical approximation of Navier-Stokes equations that are used to describe flow conditions and is commonly used since the middle of last century [2,3]. Primarily used for water flow, pressure and velocity simulations today there is no industry which does not use CFD for the modelling of different systems ranging from micro scale (flow in microreactors) to macro scale (ocean currents and weather forecast) [4]. The main problem today is which software to use since the last two decades emerged with more than a few options that are user friendly like ANSYS, Open FOAM, PowerFLOW, SimScale, Comsol Multiphysics, Autodesk CFD, FLOW-3D and others. All of the mentioned software's come with build in Navier-Stokes equations and some even have extensive libraries with prebuild materials and objects. The main drawback still is the computer speed for solving the vast number of equations simultaneously which depending on the model quality can range from few hundreds for very simple models to few millions which are used for more demanding processes. The example of one CFD simulation for the purification of biodiesel which was obtained with the use of Comsol Multiphysics software is presented in Fig. 1. In this case microdevice with two Y-shaped inlets was used, where deep eutectic solvent (DES) was introduced in upper channel and row biodiesel in lower channel at the same flow rates. Based on the calculated Reynolds numbers laminar regime of flow was observed and thus this system was described as a laminar two-phase flow system. As seen in Fig. 1a the very fine grid was used in order to get the more precise model especially at the boundaries of two phases for the calculation of velocities of two phases.

This sort of models like one presented in Fig. 1b can give insight into the problems that may occur during the experimental phase and one can test different shapes of microreactors in order to find the most suited one for certain chemical or biochemical process. As often discussed one of the main problems with working with microreactors is the clogging. By using this approach clogging can be predicted and even avoided with the use of different flow rates or microreactor shape.

More details about Navier-Stokes equations, initial and boundary conditions and the use of simplified models which can be used to describe flow in microreactors are presented below.

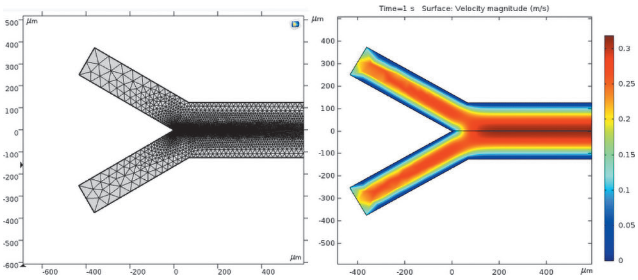

Fig. 1. CFD simulation of velocity profile in microsystem (a) grid used for the simulation, (b) velocity profile

2. Modelling of the flow in a microreactor

Microreactors are suitable for conducting processes in a single-phase and multiphase systems. When talking about reactions in multiphase systems it is important to ensure good mixing and good mass transfer of matter. In these systems the reaction rate is affected by concentration of reactants and with transfer between phases [5]. By applying micro precision engineering technologies, it is possible to construct reaction systems that ensure the intensification of mixing through effective heat and mass transfer. To ensure the optimal process conditions in a microreactor systems it is necessary to analyse the hydrodynamic and heat and mass transfer conditions.

The Navier – Stokes equation [6] can be used to describe the flow conditions in microreactors. For multiphase systems, these equations are obtained using the second Newton's laws for fluid flow [7]. If there is a gas phase in the system Navier-Stokes equation for compressible fluids is used (Eq. 1):

$$\rho\cdot\left(\frac{\partial v_i}{\partial t}+v_j\cdot\frac{\partial v_i}{\partial x_j}\right)=-\frac{\partial p}{\partial x_i}+\rho\cdot g_i+\frac{\partial}{\partial x_k}\left[\mu\cdot\left(\frac{\partial v_i}{\partial x_k}+\frac{\partial v_k}{\partial x_i}-\frac{2}{3}\cdot\delta_{ik}\cdot\frac{\partial v_j}{\partial x_j}\right)\right] \quad (1)$$

where v_i is the component of the flow velocity in the x direction, ρ is the fluid density, μ is the dynamic viscosity, p is the pressure, g is the acceleration of the force of gravity, t is the time and δ_{ij} is the Kronecker symbol. For the complete definition of the system, the mass conservation equation is also required (Eq. 2):

$$\frac{\partial p}{\partial t}+\frac{\partial}{\partial x_i}\left(\rho\cdot v_i\right)=0 \quad (2)$$

To describe the flow of liquid in microreactors, Navier-Stokes equation for incompressible fluids is applied (Eq. 3):

$$\frac{\partial v_i}{\partial t}+v_j\cdot\frac{\partial v_i}{\partial x_j}=-\frac{1}{\rho}\cdot\frac{\partial p}{\partial x_i}+g_i+\frac{1}{\rho}\cdot\frac{\partial}{\partial x_j}\left(\mu\cdot\frac{\partial v_i}{\partial x_j}\right) \quad (3)$$

When talking about fluid flow in pipes (as for microchannels), it is possible to simplify the Navier-Stokes equation, assuming that the flow is stationary and there is a constant pressure drop (Eq. 4):

$$q=\frac{\Delta p}{L}\cdot\frac{\pi\cdot d^4}{128\cdot\mu} \quad (4)$$

The above expression is known as the Hagen-Poiseuille flow, where L and d are the length and the diameter of microchannels. The Hagen-Poiseuille flow is characterized by maximum velocity in the middle of the microchannel and minimum velocity on the walls. According to Plazl and Lakner [8] at laminar flow conditions, the velocity profile fully developed in the least dimensions ([W, -W]) can be described as a function of y position only (Eq. 5):

$$v_x(y)=v_{max}\left[1-\left(\frac{y}{W}\right)^2\right] \quad (5)$$

2.1. Liquid-liquid flow profiles in a microreactor

Liquid-liquid two-phase systems have found application from extractions, reactive extractions, polymerizations, to multiphase catalysis [9]. Despite its great industrial importance, many details regarding the processes of mass transfer in these systems are still poorly understood. This is especially true for heterogeneous reactions in which there is a "competition" between the mass transfer and the chemical reaction [10].

Depending on the experimental conditions, several flow profiles may be formed in the liquid-liquid system in the microreactors; segmented (Taylor) flow, bubble flow, parallel flow or annular flow [11]. Segmented and parallel flow profiles are most commonly developed (Fig. 2) [12].

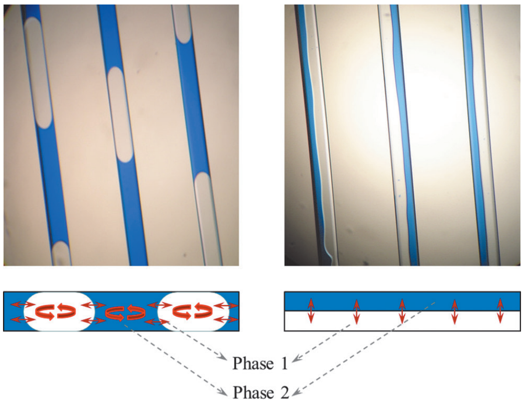

Fig. 2. Liquid-liquid system flow profiles in a microreactor

The development of segmented flow occurs by introducing two immiscible liquids into a microreactor. The main characteristic of this flow profile is a series of alternating segments of two phases, each segment being considered as a separate sub-volume. Segmented flow in the liquid-liquid system provides intense mass transfer within and between segments [13]. The mass transfer for liquid-liquid segmented flow takes place through two mechanisms: convection within a segment and diffusion between two segments [14]. The most important forces that describe segmented flow in a liquid-liquid system are most easily analysed by placing them in the ratios of dimensionless numbers (Table 1).

Jurinjak Tušek et al. [15] analysed slug flow in seven systems of organic solvents (chloroform, dichloromethane, diethyl ether, ethyl acetate, ethyl acetate, ethyl acetoacetate, hexane and toluene) and aqueous phase in a microreactor. Influence of linear flow velocity on slug lengths was explored for microreactor systems. Physical and chemical properties of the selected organic solvents were considered. To predict the slug length of both organic and aqueous phase non-linear regression model, liner regression model and artificial neural network model were proposed. The obtained results show that although quite simple and statistically not precise enough, regression models describe qualitatively the organic solvent-aqueous phase two-phase system with low accuracy. When true nonlinear models with neural networks were applied, high significance ($R^2 = 0.9$) of statistical predictions for the slug lengths were achieved.

3. Mass transfer in a microreactor

The basic equation used to describe the mass transfer in the case of incompressible fluids is derived from the general equation describing convection and diffusion (Eq. 6) [7]:

$$\frac{\partial c}{\partial t}+v_i\cdot\frac{\partial c}{\partial x_i}=\frac{\partial}{\partial x_i}\left(D\cdot\frac{\partial c}{\partial x_i}\right)-r \quad (6)$$

where c represents the concentration, v the flow rate, D the molecular diffusivity, and r the rate of the chemical/biochemical reaction. Simple analysis of the mass transfer in microchannels is performed by defining the value of Peclet's dimensionless number (Eq. 7):

$$\mathrm{Pe}=\frac{v\cdot L}{D} \quad (7)$$

Table 1. Important dimensionless numbers for describing liquid-liquid segmented flow in a microreactor [14]

Dimensionless numbers	Description
$\mathrm{Ca} = \frac{\mu \cdot v}{\sigma}$	ratio of viscous force to surface tension
$m = \frac{c_1^*}{c_2^*}$	ratio of soluble saturation concentrations in phases 1 and 2
$\mathrm{Fo} = \frac{t \cdot D}{r^2}$	ratio of real time to time needed to reach steady state
$\mathrm{Pe} = \frac{d^2 \cdot v}{D \cdot L}$	ratio of mass transfer by convection and molecular diffusion
$\mathrm{Re} = \frac{v \cdot \rho \cdot d}{\mu}$	ratio of inertial and viscous force
$D_\mathrm{R} = \frac{D_1}{D_2}$	solubility diffusivity ratio in phases 1 and 2
$\mu_\mathrm{R} = \frac{\mu_1}{\mu_2}$	viscosity ratio of soluble substance 1 and 2

For most chemical and biochemical processes, especially those taking place in multiphase systems, diffusion is a limiting step [16]. By reducing the dimensions of the reactor, the characteristic distance that the molecules must pass is reduced, leading to a significant shortening of the diffusion time. Microchannels are characterized by small values of the Reynolds numbers (values generally below 100) and flow is usually laminar. Under laminar flow conditions phase mixing depends only on the diffusion of molecules from one phase to another. Diffusion process in microchannel take place according to Fick's law which correlates the dynamic change of concentration and the product of diffusion coefficient and concentration gradient. Small molecules, characterized by higher values of diffusion coefficients, can quickly diffuse and distribute between phases even at short residence times. Opposite to that, large molecules like enzymes or whole cells slowly diffuse and remain largely in the phase at which they are introduced into the microsystem [17].

The diffusion time is particularly interesting for diffusion process in a microreactor channels. Since diffusion time is the ratio of the square of the path and the diffusion coefficient, by reducing the size of the process equipment of a microreactor results in the very short time needed for the molecule to diffuse in the process space (Fig. 3).

The application of diffusion to processes carried out in microreactors can be divided into several main groups (*i*) cell extraction from suspensions, (*ii*) mass transfer with a stable concentration gradient, (*iii*) protein deposition and (*iv*) improving fluid mixing with the aim of more intensive diffusion of solvents. When microreactors are used to investigate the kinetics of reactions, the rate of the reaction should not be affected by the mass and energy transfer in order to obtain intrinsic kinetics [18] or the effect of diffusion needs to be taken into account [19].

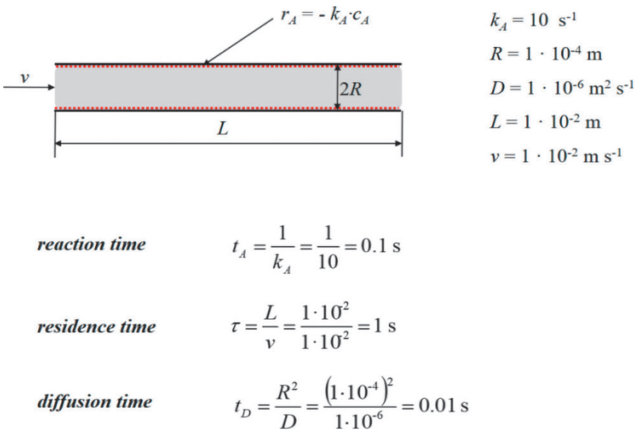

Fig. 3. Characteristic times for microreactor system

When substrate and enzyme suspension were fed through the Y-shape inlet of the microreactor formation of laminar flow in the microreactor channel was observed. The two streams, the enzyme suspension and the substrate solution, which both have a laminar flow profile, are not mixed by convection. Namely, molecular diffusion takes place between them. The substrate is a smaller molecule, so it diffuses faster into the enzyme stream, while diffusion of the enzyme into the substrate phase is much slower. The reaction takes place where both, the substrate and the enzyme are present, respectively. Hexanol oxidation catalysed by NAD^+ dependent alcohol dehydrogenase from baker's yeast in a microreactor was modelled by Tušek et al. [20] as a pseudo homogeneous process with the double substrate Michaelis-Menten rate expression. In comparison with kinetic parameters estimated in the cuvette, 30-fold higher maximum reaction rate and relatively small change in the saturation constants is observed for the kinetic parameters estimated in the continuously operated tubular microreactor. Kinetic measurements performed in a microreactor shown results without product inhibition which could be explained with hydrodynamic effects (all experiments were performed in the slug flow regime) and continuous removal of inhibiting products. Šalić et al. [21] estimated kinetic parameters of the mathematical model for the NAD^+ regeneration performed in a microreactor. The influence of acetaldehyde, ethanol, NADH and NAD^+ concentrations on the reaction rates were measured. The negative effect of both products on the reaction rate was not observed for kinetics measurement performed in a microreactor which is a consequence of investigated concentration range and continuous mode of operation. Also, Jurinjak Tušek et al. [22] estimated parameters of double substrate Michaelis-Menten kinetic model describing laccase catalysed catechol oxidation based on an individual experiment performed in a microreactor. For the estimation of kinetic parameters, the concentrations of one reactant were varied, while the second was kept constant in saturation. The estimated value for the maximum reaction rate detected in a microreactor was approximately two-fold higher than the one observed in a cuvette. This effect can be explained by taking into account the high surface area-to-volume ratio in a microreactor, and short diffusion paths that ensure fast mass transfer, and make the reaction performed in the microreactor faster.

4. Simulation of chemical and biochemical processes in a continuous flow microreactor

In order to simulate chemical and biochemical processes in a continuous flow microreactor a coupled system of convection-diffusion reaction in combination with hydrodynamics has been developed. Many studies describing mathematical simulations of microfluidic systems are available: (*i*) 3D model for description of the mass transfer of a single steroid from water into ethyl acetate considering convection in the flow direction and diffusion in all directions [23], (*ii*) 2D model of laccase catalysed L-DOPA oxidation in a microchannel considering convection in the flow direction and diffusion in two directions [24], 2D model of laccase catalysed catechol oxidation in a microreactor [22] (*iii*) 2D model of polyphenols extraction in aqueous two-phase system [25]. All mentioned models are based on the rectangular microchannel cross-section (Fig. 4).

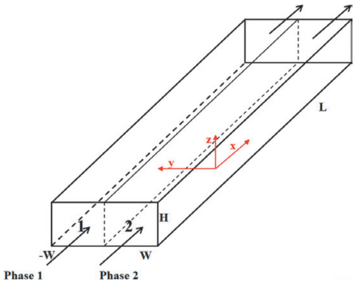

Fig. 4. Scheme of the microchannel used for development of mathematical models for different microsystems

The mathematical model for steady-state conditions in a microextractor (polyphenols extraction in aqueous two-phase system) [25] was composed of dimensionless partial differential equations for polyphenols concentrations in PEG an AMS phase and corresponding boundary and initial conditions (Eq. 8-9):

- Polyphenols concentration in PEG phase:

$$
\begin{aligned}
&v\cdot\frac{\partial\gamma_{polyphenols,PEG}}{\partial\xi}=\frac{D_{polyphenols/PEG}}{W}\cdot\left(\frac{\partial^2\gamma_{polyphenols,PEG}}{\partial\xi^2}+\frac{\partial^2\gamma_{polyphenols,PEG}}{\partial\psi^2}\right) && \\
&\gamma_{polyhenols,PEG}(0,\psi)=0 && 1\le\psi\le0 \\
&\frac{\partial\gamma_{polyhenols,PEG}\left(\frac{L}{W},\psi\right)}{\partial\xi}=0 && 1\le\psi\le0 \\
&\gamma_{polyhenols,PEG}(\xi,0)=\frac{D_{polyphenols/AMP}}{D_{polyphenols/PEG}}\cdot\frac{\partial\gamma_{polyhenols,AMP}(\xi,0)}{\partial\psi} && 0<\xi<\frac{L}{W} \\
&\frac{\partial\gamma_{polyhenols,PEG}(\xi,1)}{\partial\psi}=0 && 0<\xi<\frac{L}{W}
\end{aligned}
\tag{8}
$$

- Polyphenols concentration in AMS phase:

$$
\begin{aligned}
&v\cdot\frac{\partial\gamma_{polyphenols,AMP}}{\partial\xi}=\frac{D_{polyphenols/AMP}}{W}\cdot\left(\frac{\partial^2\gamma_{polyphenols,AMP}}{\partial\xi^2}+\frac{\partial^2\gamma_{polyphenols,AMP}}{\partial\psi^2}\right) && \\
&\gamma_{polyhenols,AMP}(0,\psi)=\gamma_{polyhenols,AMP,i} && 0\le\psi\le1 \\
&\frac{\partial\gamma_{polyhenols,AMP}\left(\frac{L}{W},\psi\right)}{\partial\xi}=0 && 0\le\psi\le1 \\
&\gamma_{polyhenols,AMP}(\xi,0)=K_P\cdot\gamma_{polyhenols,PEG}(\xi,0) && 0<\xi<\frac{L}{W} \\
&\frac{\partial\gamma_{polyhenols,AMP}(\xi,1)}{\partial\psi}=0 && 0<\xi<\frac{L}{W}
\end{aligned}
\tag{9}
$$

where ν represents linear velocity, ξ and ψ represent independent dimensionless variables $\xi = x/W$, $\psi = y/W$, x and y are coordinated in the length (L) and microchannel width ($2W$). $D_{polyphenols/PEG} = 2.37 \cdot 10^{-7}$ m^2 s^{-1} and $D_{polyphenols/AMP} = 2.20 \cdot 10^{-6}$ m^2 s^{-1} are diffusion coefficients for polyphenols in PEG and AMP phases. The molecular diffusion coefficients for polyphenols were calculated using the Hyduk-Laudie empirical correlation (Eq. 10):

$$D_{polyphenols/PEG(AMP)}=\frac{13.26\cdot10^{-9}}{\eta_{PEG(AMP)}^{1.14}\cdot V_{m,polyphenols}^{0.589}} \tag{10}$$

where $V_{m',\mathrm{polyphenols}}$ is molar volume of gallic acid as standard for polyphenolic compounds, and η is water dynamic viscosity of prepared solution of PEG (AMP).

The goal of solving listed differential equations is to find a function (or a discrete approximation of a function) and boundary conditions along the boundary of a given domain, which satisfy the given relations between different derivations in a given region of space and/or time. It is very difficult to obtain a solution that satisfies the differential equation in the whole area of consideration. Therefore, numerical methods based on the discretization of a continuous system were used where the differential equations were replaced by a system of algebraic equations. The three most common methods for numerically solving partial differential equations are: (*i*) finite difference method, (*ii*) finite element method and (*iii*) finite volume method. Jurinjak Tušek et al. [23] described the simple method for solving partial differential equation of the diffusion and convection in a microreactor. Partial differential equations were solved by approximating microreactor with two parallel plug flow reactors (Fig. 4) in order to investigate application of simple numerical approximations for solving the system of partial differential equations.

5. Conclusion

The variety and complexity of process taking place in microreactor systems pose major challenges to the modelling approaches. To precisely describe the process in micoreactors hydrodynamics, mass transfer and chemical/biochemical kinetics have to be taken into account and efficient computational tool should be used to get reliable simulation results.

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Solid-state fermentation technology and microreactor technology – Opposites that attract each other

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Abstract

Solid-state fermentation can be considered as a robust technology as being a complex system of chemically heterogeneous substrate(s) and microorganism with the difficulties to assure homogeneity of the system, with oxygen transfer limitation, heat accumulation, etc. However, higher production yield and lower economic aspect in comparison to submerge fermentation are the main force for the work on its technical enhancement, especially in the work on scale-up of the process for broader industrial purpose. Everything the opposite presents microreactor technology.

In this paper, interaction and complementarity of solid-state fermentation and microreactor technology have been presented. These technologies have been synergistically performing in the last decade, via several national and EU-funded projects, in the laboratories of the two groups from the J. J. Strossmayer University of Osijek, Faculty of Food Technology Osijek, and University of Zagreb, Faculty of Chemical Engineering and Technology.

In the first part of the paper, the origin and the chemical structure of the substrates used for solid-state fermentation and the main aspects of solid-state fermentation for different applications are presented. The basic aspects of solid-state fermentation and basic principles of solid-state bioreactors are given in second part of the paper. The third part is dedicated to the presentation of microreactors technology as a supportive and effective tool before, during and after performing solid-state fermentation. The last chapter is our vision of the future work in the development of the sustainable and effective processes of production of the valuable products from the waste materials.

Keywords: *microreactors, solid-state fermentation, lignocellulose*

1. Origin and the chemistry of the lignocellulosic substrates

The term “lignocellulosic biomass” refers to higher plants, softwood or hardwood. Therefore, it mainly origins from agricultural, food or wood industries. 52% of total land in Croatia is agricultural land. Harvest residues are usually left in the field, but with the improvement of the pretreatment process along with soil protection, they could be used for the production of huge amounts of energy in the future or for the production of the fine chemicals [1]. Brewers spent grain (beer production), grape pomace (wine production), oil pomace (oil production), sugar beet waste (sugar production) can be considered as the main lignocellulosic waste materials or by-products from food industry in Croatia. When talking about wood industry, plenty of sawdust remains during wood processing and they are among all mention lignocellulose materials the most difficult for degradation. The answer to the question *Why* lays in the complexity of the material structure of lignocellulose materials (Fig. 1) and in lignin as the most difficult biodegradable polymer. Lignin is a major barrier in lignocellulosic biomass bioconversion process and is presented in the biggest quantity in lignocellulose from the wood industry. The higher the lignin content, the greater is the resistance of the biomass to degradation. Significant efforts in the world scientific community are dedicated to the production of fine chemicals from the lignin, designing new lignin-based polymeric materials, development of new processes of microbial or enzymatic conversion of lignin, development of the sophisticated methods for lignin concentration and structure measurements, etc. [2].

The composition of agricultural, food and wood lignocellulosic biomass depends on its source, but typically it is comprised of about 40–50 % cellulose, 20–30 % hemicellulose, and 10–25 % lignin [1, 2]. Structural formula and the visual description of the complexity of lignocellulose from different industrial waste streams are presented in Fig. 1.

As already emphasized, lignin is the most complex polymer in the nature. Chemically, it is a complex aromatic and hydrophobic amorphous heteropolymer consisting of three different phenylpropane alcohols, *p*-coumaryl, coniferyl and sinapyl. Their quantity varies according to species, maturity and the space localization in the cell. Lignin gives the plant a structural rigidity, impermeability, and resistance against microbial attacks and oxidative stress. It is insoluble in water and optically inert.

Cellulose is the main component of plant cell wall and gives a plant hardness and chemical stability. It is a linear polysaccharide polymer made of long chains of cellobiose units linked via β-1,4 glycosidic linkages. In the cellulose chains a number of hydroxylic groups are presented leading to the formation of hydrogen bonds, while cellulose chains are interlinked by hydrogen bonds and van der Waals forces. Cellulose molecules can have different levels of crystallinity – low crystallinity (amorphous regions) and high crystallinity (crystalline regions). The crystalline form prevails in the major part of the cellulose and is hardly hydrolyzed in comparison to amorphous form. It is therefore expected that high-crystallinity cellulose will be more resistant to enzymatic hydrolysis, but reduction of crystallinity will increase the degradability.

Hemicellulose represents a family of polysaccharides such as pentoses (xylose and arabinose), hexoses (glucose, galactose, mannose and/or rhamnose) and acids (glucuronic acid, methyl glucuronic acid, and galacturonic acid). The dominant component of hemicelluloses from hardwood and agricultural plants is xylan, while in softwoods dominate glucomanan. Hemicelluloses have a lower molecular weight than cellulose and are more amorphous, random, and branched with little strength which makes it highly susceptible to biological, thermal, and chemical hydrolysis [1].

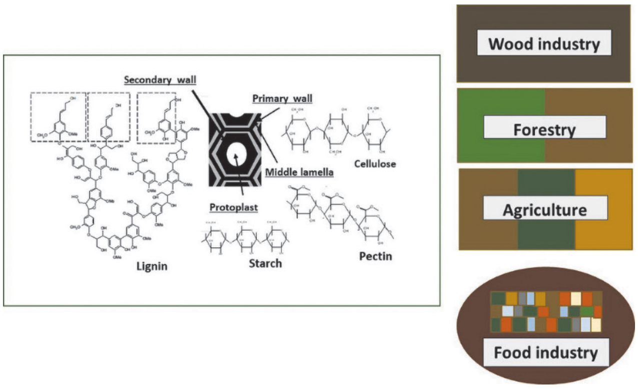

Fig. 1. Structural formula of lignocellulose with the visual description of the complexity of lignocellulose from waste streams of different industries

The main focus of our research in general is dedicated to the development of industrially important processes or products by the application of environmentally friendly technologies. We are working with the different lignocellulose substrates originated from different type of industries, mainly from food industry as well as some other waste materials (such as waste cooking oil etc.).

In Table 1, the list of our references where the utilization of the lignocellulose-type of substrates from industry are investigated is given:

2. Solid-state fermentation

Solid-state fermentation (SSF) is the method of cultivation of microorganisms on inert or non-inert solid substrate(s) under controlled conditions. Lignocellulosic materials belong to the noninert solid substrates serving as a nutrients for microorganism’s growth and metabolite production. When choosing a solid-state bioreactor, understanding of the microorganism morphology is obligatory. The process conditions (temperature, substrate humidity, initial inoculum concentration), and addition of external carbon and/or nitrogen sources, mineral compounds or specific enzyme’s inducers for microorganism’s growth and/or desired metabolite production, have to be carefully chosen [6]. The most common applied microorganisms in solid-state fermentation are fungi. Our focus is mainly dedicated to the application of white-rot fungi. The illustration of the effect of white-rot fungi during SSF is given in Fig. 2.

Table 1. Utilization of lignocellulose for different purposes

Industry	Substrate	Technology	Purpose	Reference
Agriculture	Harvest residuals	Anaerobic co-digestion with cow manure	Biogas production	[1]
	Harvest residuals	Anaerobic co-digestion with cow manure (Thermal pretreatment of lignocellulose)	Biogas production	[3]
	Harvest residuals	Anaerobic co-digestion with cow manure (Lignocellulose pretreatment by electroporation of lignocellulose)	Biogas production	[4]
	Corn silage/ forage	Anaerobic co-digestion with cow manure (Lignocellulose pretreatment by solid-state fermentation)	Biogas production	[5]
	Corn silage/ forage	Solid-state fermentation	Phenolic compounds production, enzyme production	[6-8]
	Corn silage/ forage	High-pressure and temperature extraction	Phenolic compounds production	[9]
Food	Grape pomace	Conventional extraction	Phenolic compounds extraction	[10]
	Grape seed	Conventional extraction	Phenolic compounds extraction	[11]
	Brewers' spent grain	Anaerobic digestion	Biogas production	[12]
	Brewers' yeast	Anaerobic digestion	Biogas production	[13]
	Brewers' spent grain	Solid-state fermentation	Laccase production, polyphenolic production	[8]
	Sugar beet pulp	Solid-state fermentation	Determination of pentosans by 5 different fungi	[14]
	Sugar beet pulp	Solid-state fermentation	Decolorization of dyes	[15]
Wood	Oak, ash and hornbeam sawdust	Submerge fermentation with *Trametes versicolor*	Laccase production	[16]
Other industry (including non lignocellulose materials)	Solid wet waste from the paper industry, three different commercially bleached kraft pulps	Submerge fermentation with *Trametes versicolor*	Laccase production	[16]
	Recycled grease trap waste	Esterification with and without co-solvents	Biodiesel production	[17]
	Waste cooking oil	Enzymatic transesterification	Biodiesel production	[18]
	Whey and cow manure	Anaerobic co-digestion	Biogas production	[19] [20]

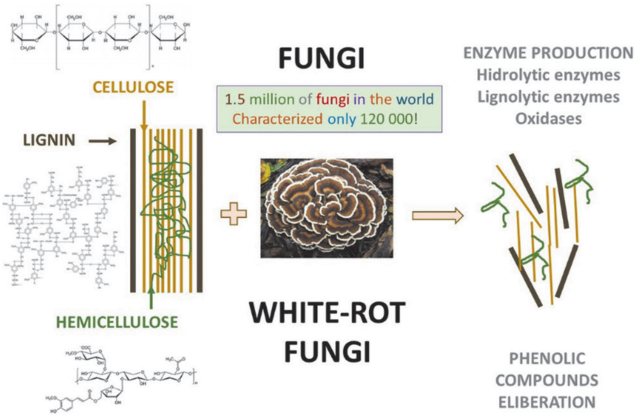

Fig. 2. The basic principle of biodegradation of lignocellose by white-rot fungi

SSF may be carried out in different types of bioreactors such as tray bioreactors, rotating disc reactors, packed-bed bioreactors, column-tray bioreactors, air-pressure pulsation solid-state bioreactors, rotating horizontal drum bioreactors, stirred-drum bioreactors, fluidized bed bioreactors, air-lift bioreactors and immersion bioreactors [2].

Photos of solid-state bioreactors for the treatment of lignocellulose are presented in the Fig. 3a (Tray bioreactor) and 3b (Horizontal bioreactor with mechanical mixing).

Tray bioreactors represent the simplest SSF technology. They are consisted of a thermostated chamber with flat perforated trays where humidified air is circulated, or water is sprayed to keep the atmosphere near saturation. They can be built from different materials such as wood, bamboo, wire or plastic. Their advantages are very simple technology and low investment cost, but when transferring into to industrial level significant problems occur, such as bed loading and large areas requirements are needed, scalable by numbers (great number of trays are needed), they are cumbersome to handle, highly labor-intensive, etc. [2].

Tray bioreactor from the Faculty of Food Technology Osijek, presented in Fig. 3a is made of stainless steel and has dimensions of 75 x 154 x 70 cm. It is consisted of six trays (50 x 5 x 40 cm) incorporated in the thermostatic chamber (25 – 65 °C) allowing the air circulation around the trays. The overall temperature of the bioreactor is controlled with 7 temperature probes (one per each plate and one for the measurement of the air temperature in the chamber) connected to PLC system. Compressed sterile

Fig. 3. a) Tray bioreactor, b) Horizontal bioreactor with mechanical mixing

air is injected directly to the fan settled inside the reactor allowing evenly air distribution with the regulation of the airflow (0.5 – 3 dm^3 min^{-1}). Additional container with water is used for moisturizing the air [2].

Another bioreactor from the Faculty of Food Technology Osijek is horizontal stainless steel bioreactor with mechanical stirring, with double walls and has total volume of 19 L. It is equipped with window glass for visual monitoring of the material with LED diodes. Stirring is performed by mechanical stirring with the possibility to regulate the speed from 1 to 50 min^{-1}. It has possibility to regulate time of stirring and non-stirring period. It is settled on the vibration table which has vibration on/off mode. The purpose of vibration is mainly for the easier sampling during the fermentation time. The port for the material sampling is placed on the bottom of the bioreactor. Three temperature probes are located on the top of the reactor. Aeration is performed with sterile air with the possibility of air-flow regulation (1-10.5 L/min). Bioreactor is equipped with the additional graduated tank for the water and liquid substrate addition. Sterilization is performed *in-situ*.

There are many works done on the application of SSF process at laboratory-scale for producing different metabolites but only a few have been published where the scale-up of the process is used and explained in details. Recent researches of solid-state fermentation for the production of enzymes, phenolic compounds or as a pretreatment method for biogas production, done by our group is presented in Table 2.

Phenolic compounds have been recognized for their influence on human metabolism and in prevention of some chronic disease and being good antioxidants in food. Usually, chemical synthesis or conventional extraction are used for producing phenolics from natural sources [21], but solid-state technology in that purpose is finding its place among several groups in the world.

Table 2. Utilization of lignocellulose as substrates in SSF for different applications

Substrate	Microorganism	Application	References
Corn silage	*Trametes versicolor*	Production of caffeic acid, vanillic acid, *p*-hydroxybenzoic acid, and syringic acid	[7]
		Laccase production	[7]
Brewers' spent grain	*Trametes versicolor*	Total polyphenolics	[8]
		Laccase production	[8]
Corn silage	*Trametes versicolor*	Pretreatment for biogas production	[5]
Cold-press oil cakes	*Trametes versicolor* *Humicola grisea*	Nutritionally enriched product	[22]
Cold-press oil cakes	*Thermomyces lanuginosus*	Lipase production	[23]

3. Microreactors

New trends in the world market of fine chemical production are to switch from the batch process to the continuous, *flow* process. The most commonly used expression is *flow chemistry*.

Biotransformation in microreactors are described in details in previous papers via several important scientific results that gave a contribution for the development of faster, cleaner and easier biotransformation processes thanks to the microreactor technology. There are several basic supports that microreactors can offer to the solid-state fermentation technology:

1) Research on the model solution of the substrates and enzymes in order to get more in-depth knowledge on the enzymatic reactions that occur during biotransformation of lignocellulose by the whole cells of microorganisms
2) Measurement of reaction rate kinetics using model solutions in microreactors
3) Enzymes produced by solid state fermentation can be tested as biocatalyst in microreactors in the fast screening in order to find suitable substrate/enzyme system
4) Phenolic compound(s) produced after solid-state fermentation can be tested as substrates for commercial or produced (crude or purified) enzymes in microreactors in the fast screening in order to find suitable substrate/enzyme system

Here, we are presenting the results of model solution of phenolic compounds removal or degradation by enzymes in microchannels (Table 3). The first reaction in that sense was the investigation of L-DOPA oxidation catalyzed by laccase where the superiority of the microreactor process over batch process was strongly emphasized [24].

Table 3. Phenolic compounds biotranformations in microreactors

Substrate	Enzyme	Reference
L-DOPA	Laccase	[24]
Catechol, L-DOPA	Laccase	[25]
Catechol	Immobilized laccase	[26]

4. Future prospective

In order to change chemical routes of the production of some compounds with the biochemical routes, enzymes of the high selectivities and productivities have to be used. Here, the first connection of SSF with microreactors is visible: production of enzymes in cheap and ecologically friendly manner by the application of solid-state fermentation and then, the use of produced enzymes as biocatalysts in biotransformation processes in microreactors.

The other vision of ours is to liberate phenolic compounds that are entrapped in the lignocellulose matrix by the application of SSF, to isolate this compound and to perform biotransformation in microreactors with the enzyme produced by SSF.

The vision of our future work is presented in Fig. 4.

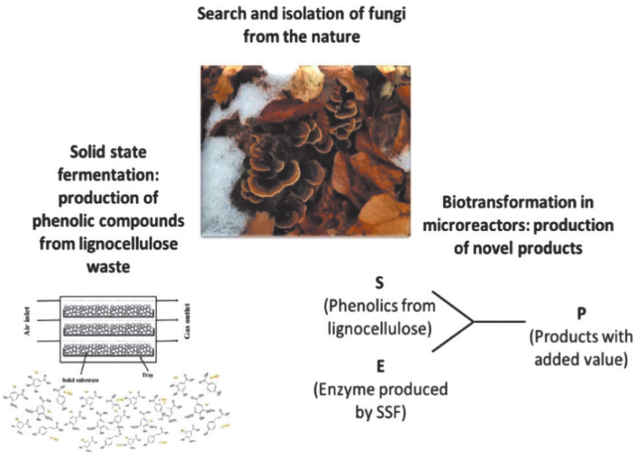

Fig. 4. Future prospective – synergy of solid-state fermentation and microreactors

Acknowledgments

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Production of microreactor systems by additive manufacturing technology

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Abstract

Microreactor systems are reactors with three-dimensional structures which are under a millimeter in size. They are commonly fabricated by wet and dry etching, precision machining, laser treatment, blasting and lithographic techniques. Additive manufacturing technologies have been overlooked in this area. This paper presents a part of research related to fabrication of microstructured reactors (microreactors and millireactors) by using two additive manufacturing technologies (fused filament fabrication and stereolithography). One example of static mixer used in a millireactor and one reactor designed for uniform droplet production are also presented.

Keywords: *microreactor, millireactors, additive manufacturing, fused filament fabrication, static mixers*

1. Introduction

Compared to conventional reactors, microreactor systems are significantly smaller in size. Microreactor systems are generally described as reactors with three-dimensional structures, the inner dimensions of which are under a millimeter in size, but usually between 10 and 100 micrometers [1-3]. Also, sometimes they are divided according to the dimensions of the internal structural units into nanoreactors (1 nm to 100 nm), microreactors (100 nm to 1 mm) and millireactors (1 mm to 10 mm) [4].

When conducting reactions in such small systems that are several orders of magnitude smaller than conventional reactors, the diffusion path is very short, resulting in intense mass and energy transfer, causing numerous positive effects such as higher conversions and fewer byproducts.

Microreactors are most commonly made by wet and dry etching, precision machining, laser treatment, blasting and lithographic techniques. Additive manufacturing (3D printing) technologies have, up till few years ago, been overlooked in this area due to a perceived limitation of resolution. Additive manufacturing technologies, especially fused filament fabrication printers, have become widely available, enabling rapid and easy prototyping and small-scale production of prototypes and objects. By using additive manufacturing technologies, the whole manufacturing process, from microreactor design in a CAD (computer-aided design) program (Fig. 1) to design and usage, can take only a few hours and microreactors with different microchannel geometries can be easily and quickly manufactured and studied. The paper will describe part of research related to microstructured reactors fabrication and design of static mixers, but also how chemical compatibility of materials was studied.

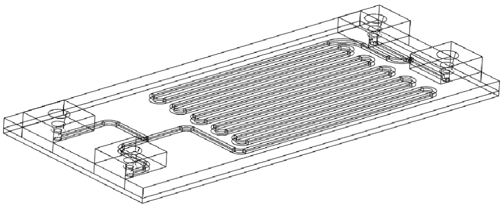

Fig 1. CAD model of the microreactor

2. Manufacturing of microreactors

The Zortrax M200 printer and the Z-Glass transparent material were used to manufacture microreactors by fused filament fabrication technology. The resulting microreactors were not completely transparent. It was found that with the maximum fill settings in the model preparation program, the fill was incomplete, leaving the air between layers, causing a loss of transparency. In addition, incomplete filling causes leakage in microreactors, i.e. liquid spills in the mass of microreactor (Fig. 2).

Fig 2. Microreactor manufactured on Zortrax M200 printer, visible penetration of blue colored fluid into microreactor mass between the layers

By properly selecting the design parameters, microreactors that do not leak and are transparent have been successfully manufactured. This was achieved primarily due to the complete filling of the mass around the microchannels and very good adhesion between polymer layers. Settings which had to be tweaked were the speed of fabrication, and the nozzle temperature which had to be slightly above the recommended temperature in order to achieve a better adhesion of the layers that will not leak. It is also important that the substrate is completely aligned with the nozzle, i.e. that there is no variation in the distance between the nozzle and the substrate when creating a single layer. The proper distance of the nozzle from the substrate at the beginning of construction is also important. If the nozzle is too close to the substrate, the buildup of the following layers will cause excess material to build up, which can clog the duct. If the nozzle is too far from the substrate, there will be insufficient material and no good contact between the polymer lines and the layers will occur, causing poor transparency and leakage inside the microreactor. It has been observed that microreactor transparency is a good indicator of whether the resulting microreactor will leak. If the microreactors are transparent and do not have visible lines of polymeric material they will not leak (Fig. 3). Fig. 3 to 5 show 3D-printed microreactors, Fig. 5 shows a microreactor channels with diameter 0.49 mm.

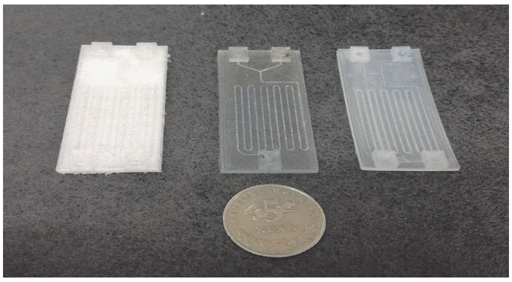

Fig 3. Comparison of microreactor transparency; a) Microreactor made of Z-Glass material on a Zortrax M200 printer, b) Microreactor made of Z-Glass material on a home-made printer, c) Microreactor made of Tough material on a home-made printer

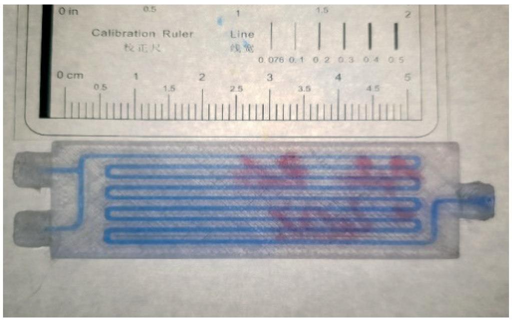

Fig 4. Manufactured microreactor and reference scale for determining microchannel width

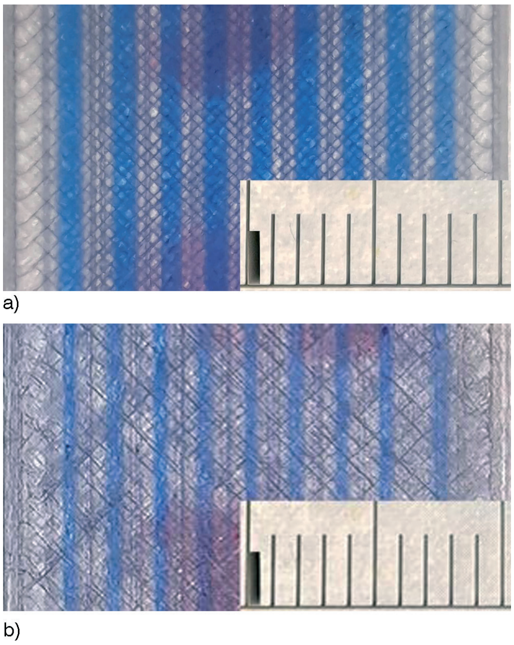

Fig 5. Measuring the channel width of a 3D-printed microreactor, diameter is a) 0.86 mm and b) 0.49 mm (scale is 10 mm).

3. Chemical compatibility of filaments

Transparent filaments of various polymers are commercially available that can be used to make microreactors. Chemical compatibility of filaments in contact with reactants and synthesis products is an important property that must be tested before using the material and fabricating the microreactor. Otherwise, dissolution, swelling, chemical reaction, or loss of material transparency may occur during the reaction. Chemical resistance of polymer materials is commonly tested with the swelling test. In this test, polymer material is immersed in different solvents during defined period. Before the test, material sample is weighted, to define its mass before exposure to solvents. During the test, material sample is weighted several times and those values are compared with initial mass of the sample [5, 6].

Swelling test was performed with four transparent polymer materials (Z-Glass, Tough, ABS-T and PLA) that can be used for microreactor manufacturing. Six different solvents were used. Redistilled water, ethanol and acetone were chosen as standard solvents that are commonly used for various applications. To test their compatibility with filaments, biodiesel, sunflower oil and a mixture of methanol and potas-

Table 1. Results of swelling test [7, 8]

Filament	Solvent	Mass increase after		Comment
		3 h [%]	6 h [%]	
PLA	water	0.4	0.4	/
	ethanol	0.4	1.3	/
	acetone	/	/	dissolved
	biodiesel	0.6	0.5	/
	sunflower oil	-0.1	-0.1	/
	methanol + KOH	/	/	dissolved
ABS-T	water	0.6	0.6	/
	ethanol	1.1	2.2	/
	acetone	/	/	dissolved
	biodiesel	21.1	30.3	lost transparency
	sunflower oil	-0.2	0.0	/
	methanol + KOH	7.3	9.8	lost transparency
Tough	water	0.0	0.0	/
	ethanol	0.0	0.0	/
	acetone	0.6	1.9	/
	biodiesel	6.6	9.9	/
	sunflower oil	0.6	0.4	/
	methanol + KOH	0.5	0.5	/
Z-Glass	water	0.0	0.0	/
	ethanol	0.0	0.0	/
	acetone	19.9	19.9	lost transparency
	biodiesel	1.1	1.4	/
	sunflower oil	-0.2	1.0	/
	methanol + KOH	/	/	dissolved

sium hydroxide (KOH) were used as specific product and reactants during biodiesel synthesis. Mass concentration of KOH in methanol was 36,12 g/L. Used biodiesel was produced via batch reaction and it was not purified. For the test, pieces of filaments (about 3 cm in length) were weighted and placed in vials with solvents. During the test with biodiesel synthesis components, vials were kept in a laboratory water bath at the temperature of 60 °C. The swelling test with redistilled water, ethanol and acetone was conducted under room conditions. Material samples were dried and weighted after 3 and 6 hours of immersion in all of the tested solvents. The mass values of the samples thus obtained were used for calculation of mass increase during the test. Results of the performed swelling test are shown in Table 1.

4. Effect of surface treatment on droplet formation in microreactors

Microreactors are increasingly used, and the main reasons for this are the small volumes of reagent consumed during the reaction and the rate of reaction carried out within the microreactor. In addition, reactions within liquid droplets are being increasingly investigated today, for which microreactors are also being tested. In order to carry out and control the reaction and to ensure reproducibility of the results it is necessary to obtain droplets of uniform size and shape.

The formation of droplets depends on the instability of the liquids and their surface tension. In passive microfluidic systems, the introduction of one immiscible fluid (dispersed phase) into another (continuous phase) leads to droplet formation by displacement, dripping, erupting or flowing

All these methods, except extrusion, are the result of capillary instability. Capillary instability is the effort of a fluid to minimize surface tension. The formation of droplets within a microreactor can be influenced by the introduction of force (electrical, magnetic or centrifugal) or by changing the material properties or the flow rate within the microreactor [9].

One of the key factors defining droplet shape is the channel geometry. In our study, the formation of droplets in channels perpendicular to each other was examined. For reactors with channel diameters of 1 mm to 2 mm, different flow rates were used (oil : water; in µL/min): 1000 : 100: 1000 : 200: 1000 : 400; 1000 : 500 for the reference sample, and additionaly 500 : 100, 500 : 500 and 500 : 50 for reactors with hydrophobized channels. Our research has shown that the size, shape and stability of droplet production is influenced by channel size, surface pretreatment (hydrophobization), and fluid flow rate.

Fig. 6a shows the flow instability in the untreated channel, while by treatment of the channels with a hydrophobic agent the flow becomes stable with the droplets in the outlet channel being regular. From all of the above, it is concluded that the treatment of the channels with hydrophobic treatment in some channels increases the number of droplets that become more regular (uniform in size). In some, however, it reduces the number of droplets, which become elongated. Flow rate also significantly affects the appearance and number of droplets in the channels.

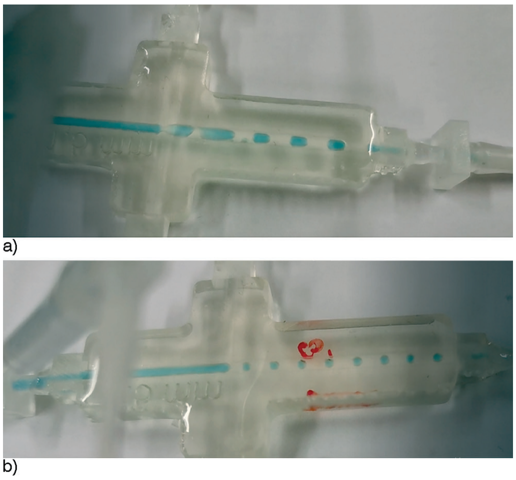
a)
b)

Fig. 6. Comparison of the number and shape of the droplets inside the channels (diameter of inlet 1.5 mm, outlet 2.0 mm), a) without treatment, b) treated with hydrophobic agent. Measured at flows: oil 1000 µL/min; water: 400 µL/min [10]

5. Addition of static mixers to millireactors

The idea of moving from batch to continuous processes is gaining interest in the industry since they allow for economical production and the inflow of larger quantities of input currents. Continuous production reduces energy consumption and waste production compared to the equivalent amount of product produced by the batch method. As many of these processes rely on good mixing and heat transfer, static mixers are being increasingly incorporated into process systems [11, 12]. Since mixing directly affects the efficiency of the process and the amount of released by-products, it is very important to characterize mixing in industrial processes for economic and environmental reasons. Static mixers, due to a series of fixed elements, redistribute fluid flow in directions transverse to the main flow. With this mixing method, mass transfer is occurring by convection rather than diffusion [13-16].

In our study [17], the effect of static mixers on the reaction conversion was examined. Fenton oxidation of organic pollutants (dye Reactive Blue 182) was studied. Millireactors with static mixers were fabricated by stereolithography (one of the additive manufacturing technologies). The millireactor used as a benchmark was a simple tubular millireactor, with 2 mm circular diameter. Several types of static mixers were designed and tested. The geometry of the millireactor with static mixers shown on Fig. 7 was in the form of a cylinder inside the cylinder with the inlet and outlet on opposite sides, which resulted in centrifugal mixing of the fluid. The flow rates used were between 100 µL/min and 2600 µL/min.

The values of Reynolds' numbers calculated for the tubular millireactor suggest laminar flow for all used flow rates. During the reaction, the laminar flow was clearly visible at all lower flow rates, while at higher flow rates,

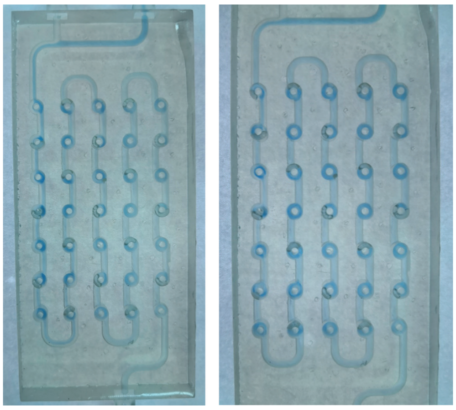

Fig. 7. Comparison of millireactors with centrifugal mixing while using low flow rates (200 µL/min, left picture) and high flow rates (2600 µL/min, right picture). Stronger mixing at the contact point of two reactant currents is noticeable on the right picture showing higher flow rate.

strong mixing at the contact point of two reactant currents was visible as shown in Fig. 7.

6. Conclusions

We have demonstrated the usefulness, feasibility and flexibility of additive manufacturing in the fabrication of microreactor systems. Within the timespan of one or two days, a microreactor geometry tailored to a specific reaction can be designed, manufactured, and used to perform chemical reaction. Due to the time and cost-effectiveness of this type of fabrication technique, it is possible to study several materials engineering and chemical engineering problems at the same time.

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