Marina Tišma[1], Mirela Planinić[1], Ana Bucić-Kojić[1], Darijo Šibalić[1], Gordana Šelo[1], Anita Šalić[2], Bruno Zelić[2]

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

[1]J. J. Strossmayer University of Osijek, Faculty of Food Technology Osijek, Osijek, Croatia
[2]University of Zagreb, Faculty of Chemical Engineering and Technology, Zagreb, Croatia

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].

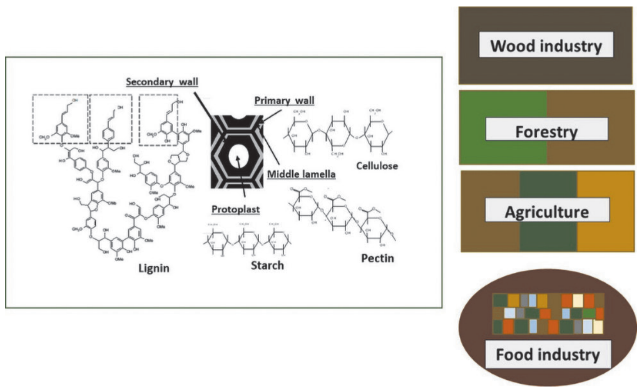

Fig. 1. Structural formula of lignocellulose with the visual description of the complexity of lignocellulose from waste streams of different industries

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]

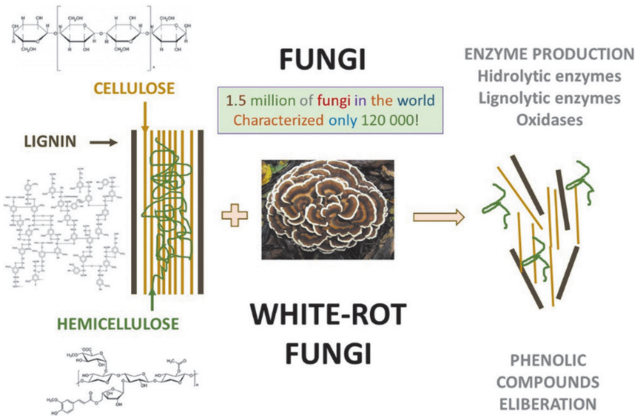

Fig. 2. The basic principle of biodegradation of lignocellose by white-rot fungi

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.

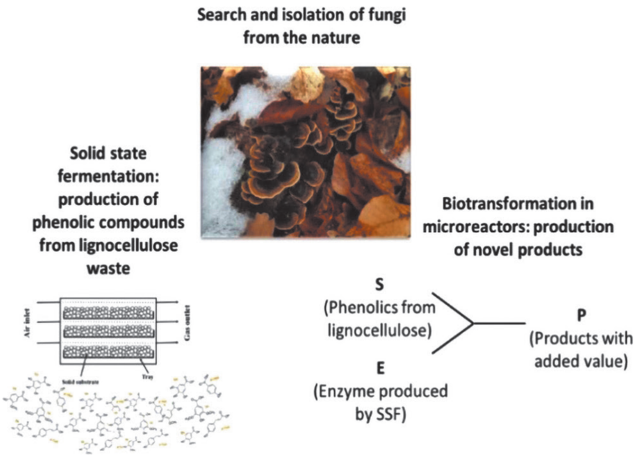

Fig. 4. Future prospective – synergy of solid-state fermentation and microreactors

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References

[1] Kovačić, Đ., Kralik, D., Rupčić, S., Jovičić, D., Spajić, R., Tišma, M. Soybean straw, corn stover and sunflower stalk as possible substrates for biogas production in Croatia: A review. Chem. Biochem. Eng. Q. 31 (2017) 187-198

[2] Isikgor, F. H., Becer, C. R. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polym. Chem. 6 (2015) 4497-4559

[3] Kovačić, Đ., Kralik, D., Jovičić, D., Rupčić, S. Popović, B., Tišma, M. Thermal pretreatment of harvest residues and their use in anaerobic co-digestion with dairy cow manure. Appl. Biochem. Biotechnol. 184 (2018) 471-483

[4] Kovačić, Đ., Kralik, D., Rupčić, S., Jovičić, D., Spajić, R., Tišma, M. Electroporation of harvest residues for enhanced biogas production in anaerobic co-digestion with dairy cow manure. Bioresour. Technol. 274 (2019) 215-224

[5] Tišma, M., Planinić, M., Bucić-Kojić, A., Panjičko, M., Zupančič, D.G., Zelić, B. Corn silage fungal-based solid-state pretreatment for enhanced biogas production in anaerobic co-digestion with cow manure. Bioresour. Technol. 253 (2018) 220-226

[6] Planinić, M., Zelić, B., Čubel, I. Bucić-Kojić, A., Tišma, M. Corn forage biological pretreatment by *Trametes versicolor* in a tray bioreactor. Waste Manage. Res. 34 (2016) 802-809

[7] Bucić-Kojić, A., Šelo, G., Zelić, B., Planinić, M., Tišma, M. Recovery of phenolic acids and enzymes production from corn silage biologically treated by *Trametes versicolor*. Appl. Biochem. Biotechnol. 181 (2017) 948-960

[8] Tišma, M., Jurić, A., Bucić-Kojić, A., Panjičko, M., Planinić, M. Biovalorization of brewers' spent grain for the production of laccase and polyphenols. J. Inst. Brew. 124 (2018) 182-186

[9] Kuzmanović, M., Tišma, M., Bucić- Kojić, A., Casazza, A.A., Paini, M., Aliakbarian, B., Perego, P. High–pressure and temperature extraction of phenolic compounds from corn silage. Chem. Eng. Trans. 43 (2015) 133-138

[10] Planinić, M., Aliakbarian, B., Perego, P., Greganić, K., Tomas, S., Bucić-Kojić, A. Influence of temperature and drying time on extraction yield of phenolic compounds from grape pomace variety "Portogizac". Chem. Biochem. En. Q. 29 (2015) 434-350

[11] Bucić-Kojić, A., Sovová, H., Planinić, M., Tomas, S. Temperature-dependent kinetics of grape seed phenolic compounds extraction: experiment and model. Food Chem. 136 (2013) 1136-1140

[12] Panjičko, M., Zupančič, G.D., Fanedl, L., Marinšek Logar, R., Tišma, M., Zelić, B. Biogas production from brewery spent grain as a mono-substrate in a two-stage process composed of solid-state anaerobic digestion and granular biomass reactors. J. Cleaner Prod. 166 (2017) 519-529

[13] Zupančič, G.D., Panjičko, M., Zelić, B. Biogas production from brewery yeast using and anaerobic sequencing batch reactor (ASBR). Food Technol. Biotechnol. 55 (2017) 187-196

[14] Bénes, I., Velić, N., Planinić, M., Šmogrovičová, D., Tišma, M. Utilisation of pentosans from sugar beet pulp by different white-rot fungi. Int. Proc. Chem., Biol. Environ. Eng. 50 (2013) 94-98

[15] Tišma, M., Komar, M., Rajić, M., Pavlović, H., Zelić, B. Decolorization of dyes by *Aspergillus ochraceus* cultivated under solid state fermentation on sugar beet waste. Chem. Eng. Trans. 27 (2012 145-150

[16] Tišma, M., Žnidaršič-Plazl, P., Vasić-Rački, Đ., Zelić, B. Optimization of laccase production by *Trametes versicolor* cultivated on industrial waste. Appl. Biochem. Biotechnol. 166 (2012) 36-46

[17] Tran, N.N., Tišma, M., Budžaki, S., McMurchie, E.I., Morales Gonzalez, O.M., Hessel, V. Ngothai, Y. Scale-up and economic analysis of biodiesel production from recycled grease trap waste. Appl. Energy 229 (2018) 142-150

[18] Budžaki, S., Šalić, A., Zelić, B., Tišma, M. Enzyme catalyzed biodiesel production from edible and waste cooking oil. Chem. Biochem. Eng. Q. 29 (2015) 329-333

[19] Hublin, A., Ignjatić Zokić, T., Zelić, B. Optimization of biogas production from co-digestion of whey and cow manure. Biotechnol. Bioprocess Eng. 17 (2012) 1284-1293

[20] Hublin, A., Zelić, B. Modelling of the whey and cow manure co-digestion process. Waste Manage. Res. 31 (2013) 353-360

[21] Maderia Jr, J.V., Barroso Teixeira, C., Alves Macedo, G. Biotransformation and bioconversion of phenolic compounds obtainment: an overview. Crit. Rev. Biotechnol. 35 (2015) 75-81

[22] Budžaki, S., Strelec, I., Krnić, M., Alilović, K., Tišma, M., Zelić, B. Proximate analysis of cold-press oil cakes after biological treatment with Trametes versicolor and *Humicola grisea*. Eng. Life Sci. 18 (2018) 924-931

[23] Tišma, M., Tadić, T., Budžaki, S., Ostojčić, M., Šalić, A., Zelić, B., Tran, N.N., Ngothai, Y., Hessel, V. Lipase production by solid-state cultivation of *Thermomyces lanuginosus* on by-products from cold-pressing oil production. Processes, 7 (2019) 465

[24] Tišma, M., Zelić, B., Vasić-Rački, Đ., Žnidaršič-Plazl, P., Plazl, I. Modelling of laccase-catalyzed L-DOPA oxidation in a microreactor. Chem. Eng. J. 149 (2009) 383-388

[25] Jurinjak Tušek, A., Tišma, M., Bregović, V., Ptičar, A., Kurtanjek, Ž., Zelić, B. Enhancement of phenolic compounds oxidation using laccase from *Trametes versicolor* in a microreactor. Biotechnol. Bioprocess Eng. 18 (2013) 686-696

[26] Jurinjak Tušek, A., Šalić, A., Zelić, B. Catechol removal from aqueous media using laccase immobilized in different macro- and microreactor systems. Appl. Biochem. Biotechnol. 182 (2017) 1575-1590