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Original paper

Simulation model comparison of submerged and solid-state hydrolytic enzymes production from wheat chaff

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Abstract Hydrolytic enzymes have a huge role in the ethanol industry since second generation (cellulosic) agro-industrial wastes have been used as raw materials for these processes. Submerged fermentation for enzymes production is widely used at the moment, but solid-state cultivation could become a cheaper process in the future. With this in mind, the purpose of this work was to compare the two aforementioned techniques by using experimentally obtained data about the fermentation stage in order to compare the two production processes through simulation. Although the experiments showed that more enzymes are being produced by the submerged cultivation method, the comparison of the two processes (submerged and solid-state) through simulation showed that the solid-state technique has better economic parameters. The profitability analysis showed that the solid-state enzyme production could be an economically reasonable solution.

Keywords Agro-industry, biomass, fungi, hydrolytic enzyme, solid-state, waste.

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Introduction

Fossil fuels are becoming a growing problem concerning their availability and also in terms of greenhouse gas emissions and global warming due to their progressive consumption. A key role in solving these problems could be found in renewable energy sources (S. SOIMAKALLIO et al, 2011 [1]). In the last decade, the most important commercial solutions represent corn ethanol in the USA, sugar cane ethanol in Brazil and rapeseed biodiesel in Germany and France (K. ARAÚJO et al, 2017 [2]). In other words, renewable energy has been employed as a potential substitute for energy derived from petroleum, i.e. fossil transport fuels (M.E.D. OLIVERIA et al, 2005 [3]). However, ethanol produced from sugar and starch caused a price increase of food on the market. This is the reason why researches of today are focused on obtaining ethanol from lignocellulose-based raw materials (M. BALAT et al, 2008 [4]).

The main challenge in producing second generation ethanol (i.e. from lignocellulosic biomass) is the transformation of complex polymers to easily adoptable sugars by the producing strain of microorganism during fermentation. These polymers are cellulose, hemicellulose and lignin, which are firmly intertwined and chemically linked with noncovalent and covalent bonds (C. SANCHEZ, 2009 [5]). Compared to starchy biomass, it is considered a very recalcitrant material because of its crystalline structure and presence of lignin.

Hence the biggest obstacle to converting lignocellulosic biomass to ethanol is its pretreatment. Because of the complex structure of these types of feedstock, pretreatment enhances their degradation, enables lignin to be removed, leads to partial or total hemicellulose hydrolysis and decreases the quantity of cellulose crystal fraction. Also, the simple sugars, which remain after hemicellulose decomposition can be converted to ethanol. Raw, untreated biomass is very resistant to enzymatic hydrolysis. Therefore, a significant number of pretreatment methods have been examined with the aim of enhancing these raw materials degradation. After pretreatment, the biomass must be subjected to enzyme hydrolysis. Obtaining an adequate enzyme or enzyme mixture for efficient hydrolysis for given lignocellulosic biomass is the key to an economically viable process of second-generation ethanol production (R. ŁUKAJTIS et al, 2018 [6]; R. ANDERSON et al, 2018 [7]; L. CHEN et al, 2018 [8]; M. SEENUVASAN et al, 2017 [9]).

Consequently, the ethanol industry urges for the development of economically viable processes for the production of hydrolytic enzymes. Regarding this, the solid-state cultivation method has its potentials, besides the widely used submerged fermentation process (L.R. CASTILHO et al, 2000 [10]). Taking this into account, wheat chaff as an agroindustry waste has the potential with its carbohydrate content besides its low price to be a suitable base compo-nent of media for enzyme production (S. RAMACHANDRAN et al, 2007 [11]).

Until now research efforts have been directed towards modelling different types of bioreactors for enzyme production or enzyme catalysis (E. GONZO and J. GOTTIFREDI, 2007 [12]; C.K. LEE et al, 2001 [13]; P. VALENCIA et al, 2010 [14]), and simulating processes for lipase production (L.R. CASTILHO et al, 2000 [10]), amylase production (A.M. DE CASTRO et al, 2010 [15]), enzyme-catalysed biodiesel production (H. YUN et al, 2013 [16]; X. ZHAO et al, 2015 [17]), bioethanol production (M. RATHNAYAKE et al, 2018 [18]; O. PARDO-PLANAS et al, 2017 [19]), hydrothermal pretreatment and enzymatic hydrolysis of lignocellulose feedstock (M.T. ASHRAF and J.E. SCHMIDT, 2018 [20]), etc. but a simulation of an entire hydrolytic enzymes (amylases, cellulases and xylanases) production process from wheat chaff has not been carried out.

That is why the aim of this work was to examine and compare the simulation models for producing hydrolytic enzymes by submerged and solid-state fermentation of wheat chaff, as well as to evaluate their economic performances.

Materials and Methods

Laboratory

Producing microorganism

For examining enzyme production, a fungi strain *Trichoderma reesei* QM 9414 has been used. Refreshing of the culture was carried out on PDA (Potato Dextrose Agar) at 28°C during 3-4 days. Before inoculation of the cultivation media, a spore suspension in sterile saline solution with 10^6 spores/g was made. Liquid media were inoculated with 10% of inoculum and the solid media were sprinkled with a defined volume of spore suspension.

Cultivation media and their preparation

Cultivation of fungi with the aim of producing enzymes was investigated with different types of cultivation methods, i.e. submerged in liquid and on solid-state media. In order to be able to compare these two cultivation methods the proposed steps by G. HANSEN et al. (2015 [21]) have been implemented in this work. The composition of the liquid media was 3 g wheat chaff, 0.5% $(NH_4)_2SO_4$ and 1.36% K_2HPO_4 in 100 mL of distilled water. For the solid-state technique, the same amount of wheat chaff (3 g) was suspended in 100 mL of a distilled water solution containing 0.5% $(NH_4)_2SO_4$ and 1.36% K_2HPO_4 like for the liquid media. After 15 min of mixing the pH value was checked and corrected to 4.5±0.1 by adding 1% NaOH or 1% H2SO4. After an additional 15 min of mixing the suspension was left to settle. The liquid phase was decanted and the residue was used as the solid media for enzyme production. In this way enzymes have been produced from the same amount of raw material used, i.e. 3 g of wheat chaff, as well as the same preparation method (100 mL of prepared salt solution), so that the obtained results could be comparable. Sterilization of the prepared media was carried out in an autoclave at 121°C and 2.1 bar for 30 min.

Cultivation conditions

Enzyme production by the submerged technique of cultivating fungi was examined in Erlenmeyer flasks of 300 mL with 100 mL of cultivation media. Cultivation

of fungi on solid media was also carried out in Erlenmeyer flasks with the same volume (300 mL) as for the liquid media. All of the experiments were carried out for 7 days at 28±1°C and in triplicate.

Analytical methods

After cultivation on solid media, the products of strain metabolism were extracted with saline solution (100 mL in order to equal the liquid volume with the submerged cultivation broths) with constant mixing at 200 rpm during 30 min at a constant temperature.

Separation of solid and liquid phase after extraction of solid media as well as the submerged cultures was carried out by filtrating through a qualitative filter paper. Obtained filtrates were subjected to standard analysis of cultivation media.

The total amount of protein in cultivation liquids and solid extracts at the end of the process was determined by the spectrophotometric Lowry method (O.H. LOWRY et al, 1951 [22]).

The intensity of hydrolytic action of the cultivation liquids and solid extracts towards starch, cellulose and xylan were assayed separately for each substrate by measuring the release of reducing sugars using the DNS (3,5-dinitrosalicylic acid) method (H.S. KIM et al, 2015 [23]). One unit (U) of enzyme activity (amylase, cellulose, and xylanase) is defined as the amount the enzyme that liberates 1 μmol of reducing sugar as glucose/mL·min under the assay conditions (N. JI et al, 2018 [24]).

Process Modelling

Given that flowsheets can be used to fully describe processes in the field of biochemical engineering (M.S. PETERS et al, 2003 [25]), the base cases and other process scenarios analysed in this work have been constructed through the use of SuperPro Designer 6.0 simulation software (Intelligen Inc., Scotch Plains, NJ, USA). Data obtained from the experimental part have been incorporated into the model, together with additional data obtained from the literature, equipment suppliers, etc.

Results and Discussion

Laboratory Experiments

In order to be able to compare the submerged and solid-state fermentation process of enzyme production, it was necessary to keep the amount of available substrate on the same level and to analyse the quality and quantity of produced enzymes in the same volume of enzyme mixture (G. HANSEN et al, 2015 [21]).

The protein content and hydrolytic activities of amylases, cellulases and xylanases after fermentation of wheat chaff media through submerged and solid-state cultivation method of *Trichoderma reesei* are presented in Table 1. Surprisingly, higher protein yield has been achieved in the submerged fermentation. This data was used to set the final protein content in the cultivation broth after fermentation in the models. As can be concluded from Table 1, there is a slight difference in cellulase and xylanase activities for the two examined cultivation methods, while amylase activity is much higher in the submerged fermentation technique. The results of the enzyme assay are comparable to previously published studies (V. PREVOT et al, 2013 [26]).

Table 1. Protein content and hydrolytic enzymes activities of cultivation broth filtrates after submerged and solid-state cultivation on wheat chaff based media

Cultivation Technique	Protein Content (mg/mL)	Amylase Activity (U/g)	Cellulase Activity (U/g)	Xylanase Activity (U/g)
Submerged	1.19 ± 0.06	187 ± 3.5	43 ± 1.6	144 ± 1.8
Solid-State	0.76 ± 0.13	133 ± 2.9	49 ± 1.2	142 ± 2.1

Modelling and Simulation

Process Model Description

The flowsheets presented in Figure 1 provides a general overview of the entire process for enzyme production by both submerged and solid-state fermentation. Production of enzymes via the submerged process, presented in Figure 1(A), starts in a laboratory flask for refreshing the producing strain *Trichoderma reesei* which is kept in a freezer.

After two days of cultivation under certain conditions, the entire contents of the shake flask are transferred to the first inoculum vessel. This vessel has previously been fed with a sterilized medium consisting of wheat chaff, water and nutrients from the medium blender. The medium blender supplies itself from the silo (with wheat chaff), water and nutrient storage. Following the cultivation in the first inoculum vessel, its content is used as inoculum for the second inoculum vessel, which is also supplied with sterilized wheat chaff based medium from the medium blender. The cultivation broth from the second inoculum vessel after the fermentation process finally has the volume and number of producing strain spores in order to be used as inoculum for the main fermenter (as it can be seen from Figure 1 the medium used for the main fermenter also comes from the medium blender).

Emissions from the laboratory (flask), inoculum preparation (first and second inoculum vessels) and fermentation (main fermenter) section that arise from cultivating the producing strain are passed through a mixer and then an air filter in order to be "cleaned" before being let out into the atmosphere. Gases from the medium blender are released directly into the air. The main fermenter (in this case liquid fermenter) output is the cultivation broth, i.e. a complex mixture of wanted products (amylase, cellulase and xylanase enzymes), formed biomass and its other metabolic by-products, residues of fermented wheat chaff

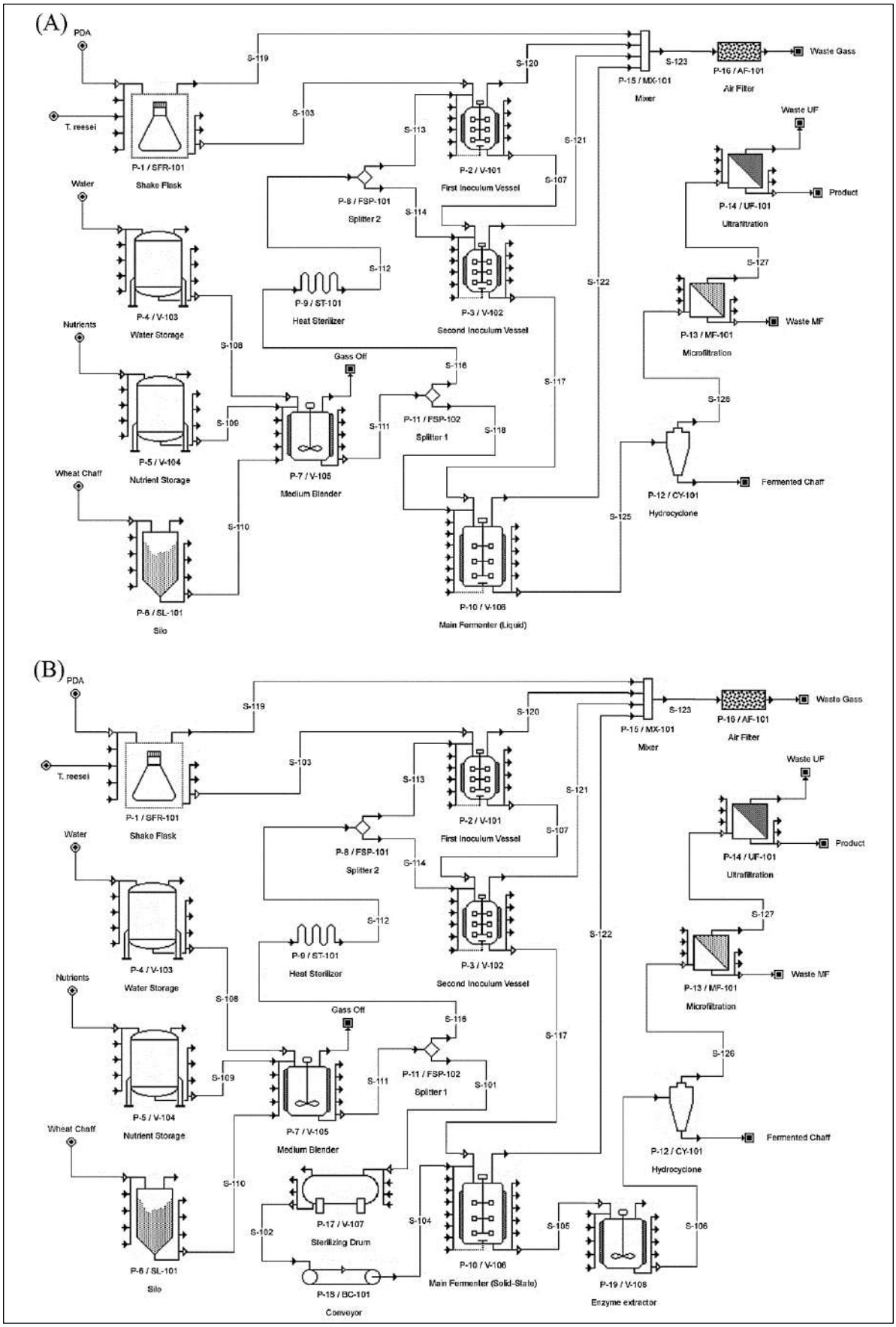

Figure 1. Simplified process flowsheet for the production of hydrolases by (**A**) submerged and (**B**) solid-state fermentation of wheat chaff obtained from SuperPro Designer.

and water. After fermentation, a hydrocyclone is used in order to separate the liquid (containing enzymes) from the solids. This separated liquid phase is first microfiltered and then ultrafiltered in order to additionally purify the final product.

As can be seen from Figure 1(B), the solid-state process differs from the submerged in two aspects. First, all of the ingredients for the main fermenter medium (wheat chaff, water and nutrients) are mixed in the medium blender and then separated by a hydrocyclone (not shown on the simplified process flowsheet in Figure 1(B) in order not the overcrowd the flowsheet, as with centrifugal pumps). The liquid part containing water and nutrient can be used again for preparing the medium in the medium blender. The solid part, i.e. the cultivation medium is transferred to a sterilizing drum in order to be sterilized.

A sterile conveyor is used to transport the sterile medium into the main fermenter, so it can be inoculated by the contents from the second inoculum vessel. Second, an additional step needs to be added in the separation stage, i.e. enzyme extraction from the cultivation broth needs to be completed after main fermentation so this mixture can be sent to the hydrocyclone.

Process Model Specifications

Plant Scale: The proposed process models are designed to be located near a local wheat milling facility in Serbia, which according to the Report of the Statistical Office of the Republic of Serbia (2017 [27]) processed 2.4×10^6 tons of wheat and obtained 10^6 tons of wheat chaff as waste. So the plant capacity for enzymes production is designed to handle this waste. By locating this plant near to the milling factory, the transportation costs of wheat chaff are negligible. Also, if the milling factory decides to invest in such an enzyme producing plant the unit production cost would be lower in terms of purchasing the raw material (wheat chaff).

Laboratory Flask: PDA medium is used for refreshing the producing strain (A.M. DE CASTRO et al, 2010 [15]). The medium is sterilized, the producing strain transferred from the frozen culture and cultivated (refreshed) as mentioned in the Materials and Methods Section. The producing strain was introduced to the software from its pure component "Biomass" in the database.

First Inoculum Vessel: Water, nutrients and wheat chaff are transferred into a medium blender (3 g/100mL wheat chaff, 0.5% $(NH_4)_2SO_4$ and 1.36% K_2HPO_4) mixed and the pH value is set to 4.5±0.1 by adding 1% NaOH or 1% H_2SO_4. After preparation, the medium is sterilized, again under conditions previously described in the Materials and Methods Section. This medium after sterilization is transferred to the first inoculum vessel, while the contents of the laboratory flask are used as inoculum for this vessel. After 48 h of fermentation (30°C and 200 rpm), the contents of the first inoculum vessel are used for inoculating the second inoculum vessel.

Wheat chaff was introduced to the software as a mixture of 54% pure component cellulose (imitating the 36% cellulose and 18% hemicellulose content in real life wheat chaff samples), 16% lignin, 4.6% protein and 6.9% ash, with a dry matter content of 91.1% according to D.H. MCCARTNEY et al. (2006 [28]). Since water, ash and protein already exist in the software database, it was necessary to create cellulose and lignin as pure components. For cellulose, a molecular weight of 162.14 g/mol per glucose unit has been used, together with a molecular formula of $C_6H_{10}O_5$ and enthalpy of -2,830.15 kJ/mol according to A.V. BLOKHIN et al. (2012 [29]). The molecular formula for lignin was set to $C_{10}H_{12}O_3$, with a molecular mass of 193.4 g/mol and enthalpy of -1,628.6 kJ/mol according to O.V. VOITKEVICH et al. (2012 [30]). The hydrolytic enzymes were introduced to the software from its database (pure component protein) with just changing the name to "active enzyme" as opposed to the pure component "inactive enzyme" which also had the characteristics of the pure component protein from the database of the software.

Second Inoculum Vessel: Substantially the order of operations is the same as for the first inoculum vessel, except for larger component quantities and equipment size.

Main Fermenter: Medium, prepared in the same way as for the first and second inoculum vessel is charged into the liquid fermenter, which is then sterilized. The entire content from the second inoculum vessel is used as inoculum for the main fermenter, which has 10^7 spores per mL or gram of raw material (wheat chaff) for liquid or solid-state fermenter, respectively. The preparation (70% moisture) and sterilization of medium for the solid-state fermenter occurs in the sterilizing drum and then it is transferred into the solid-state fermenter and inoculated. The fermentation process is carried out for seven days at 30°C and 200 rpm (mixing only for the submerged fermentation).

The fermentation process has been defined as stoichiometric and simplified according to E. JOURDIER et al. (2012 [31]). By knowing the amount of proteins produced from the experimental stage and kinetic parameters defined by E. JOURDIER et al. (2012 [31]) stoichiometric coefficients were defined for all major components taking part in the bioprocess inside the main fermenter.

Product separation and purification: The entire content from the liquid fermenter is transferred to the hydrocyclone in order to remove the solids and considering 80% of extract recovery. In the solid-state process, the fermenter content is sent to enzyme extraction with water before moving it to the hydrocyclone (the quantity of water used is calculated so as to get the same volume of cultivation broth as in the liquid fermentation process, i.e. to match the experimental part). From there, the liquid part is sent to a micro- and ultrafiltration unit. It is assumed that

the final product (ultrafiltration retentate) has a protein concentration of 50% (w/w) (A.M. DE CASTRO et al, 2010 [15]). According to W.D. MORES et al. (2001 [32]) during microfiltration 5% of all enzymes examined in this work will be denaturated, while this number is 2% for the ultrafiltration unit.

Base Case Scenario

According to the data obtained from the experiments, the amount of raw material (wheat chaff) and medium (based on wheat chaff) needed for the submerged and solid-state process per one batch could be calculated. Therefore, it is determined that 375,000 kg of wheat chaff is needed for the liquid medium of 12,500,000 kg.

As for the solid-state process, the same amount of wheat chaff (375,000 kg) per batch needs to be used, i.e. the amount medium is then 1,620,500 kg.

The base case scenario simulation (Figure 1) has taken into account that only one piece of equipment is used for all procedures and that the only product which can be sold is the final enzyme stream. This means that the fermented cake was considered as waste. Therefore, streams "Fermented Chaff", "Waste MF" and "Waste UF" in Figure 1 were classified as waste streams and a treatment cost for disposal was set to 0.0005 $/kg for each stream. As mentioned in the Section Process Model Description the centrifugal pumps for transporting fluids are not depictured in the flowsheet but are considered in the economic analysis.

Solving the material balances and analysing throughput by the software resulted in defining the process bottleneck. With an occupation time of 172 hours, the main fermenter was identified as the limiting unit for the maximum possible batches per year. This time which is somewhat larger than 7 days is necessary for fermentation stage because of the pre- and post-fermentation phases (input of medium, sterilization, cleaning, etc.).

Since the bottleneck analysis showed that the main fermenter has the shortest rest time (unit with the largest occupancy), an additional fermentation unit with the same characteristics has been added to the model, operating in staggered mode. This reduced the time needed to complete one batch from 318 hours to 312.4 hours and increased the number of possible batches in a year from 42 to 48. As the annual amount of wheat chaff available for processing in the plant was set to a constant value, the debottlenecking provided an increase in the number of batches, which led to the possibility of using smaller equipment and 15% lower capital investment for the project.

Coproduction

Since the proposed process model is intended to be located right beside the local milling company which is selling the wheat chaff to stock breeders it is inevitable to analyse the possibility of using the fermented chaff as a coproduct. According to R.L. BELYEA et al. (2004 [33]) it has been assumed that there is a 2% increase of protein in the chaff after fermentation, which made it possible to classify this output stream as an additional revenue with a selling price of 0.281 $/kg. Since generated quantities of this output stream are quite high, the income associated with this coproduct proved to be notable in reducing the enzyme unit production cost.

Economic Analysis

Two set goals are included in the economic analysis. First one is to determine and compare the unit costs ($/kg) for producing hydrolytic enzymes by the submerged and solid-state fermentation process. The second one is to evaluate the viability of both projects (submerged and solid-state) by analysing the payback time, net present value and internal rate of return as key profitability parameters (D. VUČUROVIĆ et al, 2012 [34]). In order to obtain this data, equipment costs and operating costs need to be known.

Equipment costs

The major equipment cost for the two examined processes is defined from the offers from the equipment suppliers or taken from the literature (J. FLORA et al, 1998 [35]). If the model calculates equipment sizes that differ from the offers from the suppliers, a Rule of Six-tenths is applied (L.R. Dysert, 2003 [36]).

Table 2 shows all estimations associated with equipment cost, along with their sizes and basic values for the submerged and solid-state enzyme production processes, respectively. Equipment capacities are calculated automatically by the software, based on the set enzyme production scale.

As it can be seen from Table 2, although the solid-state process requires additional pieces of equipment (sterilizing drum, sterile conveyor and enzyme extractor) the overall equipment purchase costs is much lower compared to the submerged production process. The main difference is in the "heart" of the bioprocess, i.e. the bioreactor which is much more complex, and thus more expensive in the case of the submerged mode of process operation. On the other hand, defining the amount of water needed for enzyme extraction so the final mixture has the same volume as the cultivation broth after liquid fermentation (as in the experimental work, 100 mL) leads to an enormous extractor with a huge purchase cost. Lowering the total equipment cost for the solid-state process could be in using smaller quantities of water for enzyme extraction, but this needs to be examined and analysed at a laboratory level first.

Table 2. Specifications and costs of major equipment for the process model of submerged and solid-state production of hydrolases from wheat chaff

Unit Name	Size	Cost ($)	
		Submerged	Solid-State
First Inoculum Vessel	1.42 m^3	5,000	5,000
Second Inoculum Vessel	142.15 m^3	80,000	8,000
Medium Blender	2,000.00 m^3	250,000	250,000
Heat Sterilizer	118.57 L/h	98,000	98,000
Silo	800.00 m^3	10,000	10,000
Water Storage	1,500.00 m^3	43,000	43,000
Nutrient Storage	500.00 m^3	18,000	18,000
Air Filter	11.02 L/h	4,000	4,000
Liquid Fermenter	15,104.35 m^3	3,105,000	-
Solid-State Fermenter	2,803 m^3	-	735,000
Sterilizing Drum	2,803 m^3	-	162,000
Sterile Conveyor	20 m	-	79,000
Enzyme Extractor	15,000.00 m^3	-	1,530,000
Hydrocyclone	20,571 L/h	77,000	77,000
Microfiltration	1,947 L/h	150,000	150,000
Ultrafiltration	162 L/h	147,000	147,000
All Listed Equipment		3,987,000	3,316,000
Unlisted Equipment (0.25 x All Listed Equipment)		980,000	996,750
Total		**4,983,750**	**4,145,000**

Further, from the purchase cost of main equipment, the economic parameters for capital investment have been determined as percentages of the equipment purchase cost (J.R. KWIATKOWSKI et al, 2006 [37]) and from that direct fixed capital (DFC) has been evaluated and presented in Table 3. Lower direct fixed capital costs for the solid-state process are expected, due to the fact that all of the items in Table 3 are somehow related or expressed as a function of the equipment purchase costs, which is standard practice in process modelling.

Table 3. Direct fixed capital costs estimate for hydrolases production using the submerged and solid-state methods of wheat chaff fermentation (prices in $)

Item	Submerged	Solid-State
Equipment Purchase Costs	4,983,750	4,145,000
Installation	1,495,125	1,243,500
Process Piping	1,744,313	1,450,750
Instrumentation	1,993,500	1,658,000
Insulation	149,513	124,350
Electrical	498,375	414,500
Buildings	2,242,688	1,865,250
Yard Improvements	747,563	621,750
Auxiliary Facilities	1,993,500	1,658,000
Total Direct Costs	15,848,325	13,181,100
Engineering	3,962,081	3,295,275
Construction	5,546,914	4,613,385
Total Indirect Costs	9,508,995	7,908,660
Contractor's Fee	1,267,866	1,054,488
Contingency	2,535,732	2,108,976
Total Other Costs	3,803,598	3,163,464
Total Direct Fixed Capital Costs	**29,160,918**	**24,253,224**

Operating costs

The sum of costs related to raw materials, labour, facilities, laboratory/quality control/quality analysis represents the operating cost of one plant. In other words, the working capital has been defined as the sum of the aforementioned costs during a one-month period (30 days). Assumed costs for the raw materials in this work was taken from the Report of the Statistical Office of the

Republic of Serbia (2017 [27]). The work of J.R. KWIATKOWSKI et al. (2006 [37]) was used for defining the costs of water. The price of wheat chaff at 0.3085 $/kg, was obtained from the local milling facility for which this project is intended (this price is currently for farmers who are buying the wheat chaff as animal feed). After raw materials usage and costs analysis, it can be concluded that the submerged process uses much more water than the solid-state method, and this increases the costs for the submerged fermentation for enzyme production. The examined utilities covered by the economic analysis in the enzyme production processes are electricity, heating steam, cooling water, and chilled water. Data about the costs of utilities (cooling water, chilled water, steam and electricity) needed for the process were taken from the local market (0.08 $/MT, 0.45 $/MT, 15.00 $/MT and 0.15 $/kWh, respectively). In terms of cooling water and electricity, the solid-state process is again in advantage, because more of these utilities are needed in the submerged process, thus leading to higher operating costs. Multiplying the labour demand and labour rate and summing them for the entire process provides information about total labour costs. Labour costs were determined based on the number of employees needed to run the process at a specific time and based on three shifts during the day. According to the labour rate and labour demand mentioned before, the annual labour costs are nearly equal for both examined processes. The facility-dependent costs account for depreciation of direct fixed capital costs, equipment maintenance, insurance, local taxes, and the possible other overhead-type of factory expenses (R. HARRISON et al, 2015 [38]). Laboratory/QC/QA costs in this work (representing laboratory analysis and control) are calculated as 15% of total labour costs. Miscellaneous operating costs which are all set to zero for both examined process include research and development costs, process validation expenses and others. After defining these aforementioned cost components, the total operating costs can be calculated. For the submerged enzyme production, the annual operating cost is 30,576,000 $, while for the solid-state method the operating cost is much lower (8,231,000 $). This again is due to the fact that much more utilities are required for the submerged process. Compared to results obtained by A.M. DE CASTRO et al. (2010 [15]) the annual operating cost is almost two times lower for the solid-state process.

Unit production costs

The unit costs for both processes of hydrolytic enzyme production are obtained by dividing the annual operating cost with the annual enzyme production rate. The enzyme production rate can be calculated by multiplying the quantity of proteins (enzymes) produced per batch and the number of batches per year. By applying this mathematics, the unit production cost for using the submerged method is equal to 39.58 $/kg, while for the solid-state method it is equal to 14.35 $/kg, even though more enzymes are produced in the submerged fermentation per batch (higher concentration in the cultivation broth). A three times lower unit production cost for solid-state fermentation process for producing lipases compared to the submerged processes was reported by L.R. CASTILHO et al. (2000 [10]).

Unit costs analysis, i.e. the breakdown of the unit production cost for hydrolases production from wheat chaff using submerged and solid-state fermentation is shown in Figure 2.

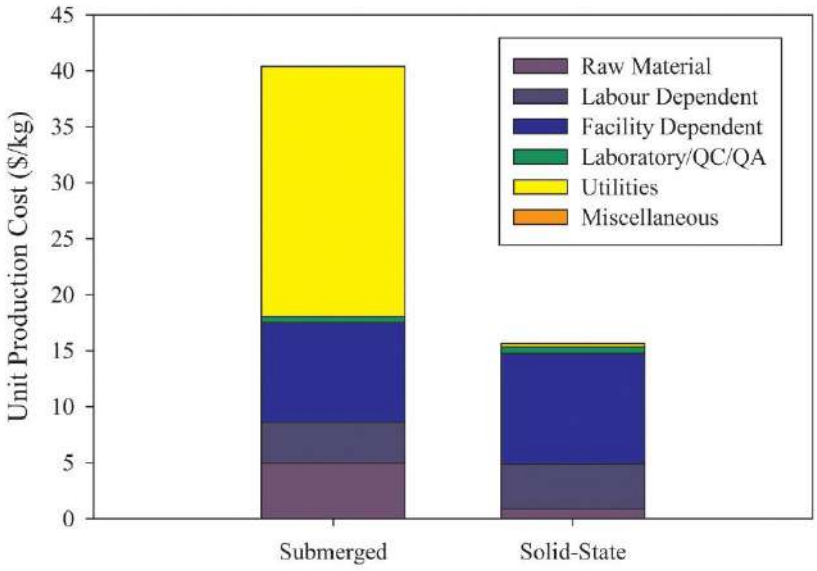

Figure 2. Breakdown of the unit production cost for hydrolases production from wheat chaff using submerged and solid-state fermentation

As can be seen in Figure 2, the major share in the unit production cost represents the expenses associated with utilities and facility dependent for the submerged and solid-state fermentation process, respectively. The second major component in the breakdown of the unit production costs is facility dependent and labour dependent items for the submerged and solid-state methods, respectively. This is only due to the fact that during the economic analysis, expenses associated with buying the wheat chaff were set to zero, as assumed that the enzyme production plant is to be built next to the wheat milling factory, i.e. it would be using its own waste (chaff) for producing another high-value product. If that were not the case, raw material costs would surely be a major part of the unit production costs. Figure 2 also indicates two other things, one of them being that the solid-state method has lower unit production costs, and the second that it has lower costs of utilities and raw materials and thus it is more economically viable.

Profitability analysis

Further profitability analysis has been carried out only for the solid-state process, due to the fact that it showed to be superior compared to the submerged method when producing hydrolytic enzymes from wheat chaff.

The time required to recover the money invested in a project is called the payback period. This payback period represents an important determinant of a given investment or project. It helps in deciding whether to invest in such a project, concerning that shorter payback periods are typically more desirable for investment opportunities. Unlike other methods of capital budgeting (net present value, internal rate of return, etc.), the payback period ignores the time value of money. It is calculated by dividing the total capital investment with the net profit and is equal to 2.75 years for the solid-state process. The net present value (NPV) is an economical parameter that tells company managers and investors to invest in a project only if it has a positive net present value. They should avoid investing in projects that have a negative net present value. At a discount rate of 7% the net present value for the solid-state process is 29,895,000 $. The internal rate of return (IRR) is a metric used in capital budgeting to estimate the profitability of potential investments. The internal rate of return is a discount rate that makes the net present value (NPV) of all cash flows from a particular project equal to zero. Generally speaking, the higher a project's internal rate of return, the more desirable it is to undertake. The IRR for the solid-state process is evaluated at 27.93%. Based on the values of the chosen indicators of profitability, the solid-state fermentation process for hydrolytic enzymes production is economically viable.

Conclusion

The present work examined and compared submerged and solid-state fermentation simulations of hydrolases production from wheat chaff at laboratory scales and using the software SuperPro Designer to model obtained results. Experimental results show that more enzymes are being produced in submerged cultivation. Enzyme activities toward a certain substrate are the same for cellulases and xylanase but are slightly higher for amylases when it comes to the submerged fermentation. The results of the simulations gave an executive and a detailed summary of the examined processes. Economic analysis showed that the solid-state process is a more attractive solution compared to the submerged. Further studies need to be carried out concerning the reuse of the liquid remaining after separation from the solid phase (which is the medium for solid-state fermentation) and examining the quantity of liquid needed to extract the enzymes from the solid-state fermentation medium after fermentation and the effect of these on process economics.

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