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Industrial Scale Bioprocess Simulation for *Ganoderma Lucidum* Production using Superpro Designer

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Abstract. The medicinal mushroom *Ganoderma lucidum* is used by traditional medicine for human infection treatments such as gastric cancer, hypertension, hepatitis, chronic bronchitis and hypocholesterolemia. However, the conventional production of *Ganoderma* in a solid phase on a large scale is costly and requires excessive processing times, which hinders its technical-economic viability. Based on the preceding, engineering studies are needed to predict their large-scale production, to identify the necessary industrial equipment and costs to propose strategies that reduce operating costs. The SuperPro Designer computational tool is a very versatile simulator used in a wide variety of industrial applications. That is why the purpose of this research is to evaluate the production of the *Ganoderma lucidum* fungus in large-scale growth culture from a computational approach. The latter, by performing simulations using SuperPro Designer software to identify process yields and propose improvements aimed at increasing productivity. The software was calibrated with experimental data reported from literature and different strategies were intended to determine the economic viability. It was found that the volume of the bioreactor significantly affects production costs compared to exopolysaccharides yields, obtaining values of 6.82 USD/g in a 2 m³ bioreactor while in a production volume of 20 m³ the costs are significantly reduced to 0.8 USD/g. The findings found here demonstrate the importance of predicting a large-scale bioprocess to improve the overall productivity of a biotechnological product.

1. Introduction

Exo-polysaccharides (EPS) are essential components produced by fungi [1]. EPS obtained by *Ganoderma lucidum* has significant functionalities. This fungus has been used by traditional medicine for the treatment and prevention of human infections such as gastric cancer, hypertension, hepatitis, chronic bronchitis and hypercholesterolemia. There is evidence that active biological components from *Ganoderma lucidum* influence beneficial treatment implied in many of the diseases [2]. *Ganoderma lucidum* is commonly known in China as "Lingzhi" and in Japan with the name of "Reishi." In these countries, it has been widely used as an ingredient. Also, *Ganoderma lucidum* has been used for foods and raw materials. The latter, due to its proven effects as a stimulator in the immune system and its anti-cancer activity [2]. This fungus is essential in Asian medicine and is known as the "king of herbs," as it contains many bioactive compounds such as therapeutics and polysaccharides. It has been used anciently by Eastern cultures for its medicinal benefits related to migraine, hypertension, arthritis, bronchitis, asthma, diabetes, hypercholesterolemia and cardiovascular problems, etc. EPS are extracellular polysaccharides released into the environment where fungi also use them as protective mechanisms.



According to Asian medicine, it has many benefits for human health, so they contain various mechanisms to attack common diseases in humans. However, more studies are needed to expand knowledge about these fungi in countries with high biodiversity, such as Colombia. Traditional *Ganoderma lucidum* growth in the solid phase is costly and requires excessive processing times, which hinders its technical-economic viability. The production of the *Ganoderma lucidum* in a solid phase requires excessive time to meet current market demand, so a long cultivation time of approximately six months is needed. Therefore, its traditional production is slow, economically unfeasible, and potentially the process is prone to contamination [1]. For these reasons, a strategy to technical the *Ganoderma lucidum* culturing is to replace the traditional solid-phase culture by the use of automated bioreactors that allow the submerged cultivation of various biological processes [3-5]. The above, for better control of culture conditions such as pH, stirring, temperature, and oxygen required [6-7]. During the last decades, there has been a marked increase in commercial interest in EPS products, not only in Asian countries but also in North America and Europe. The world production of this mushroom is approximately 6000 tons, and half comes from China. The latest available estimates place the annual value of *Ganoderma lucidum* products at more than US \$ 2.5 billion. [8] However, according to the authors' knowledge, there are no economic feasibility studies to identify the costs involved during the submerged cultivation of *Ganoderma lucidum* in bioreactors focused on an industrial scale. Based on the above, engineering studies are required to predict its productivity on a large scale, to identify the required industrial equipment, and to propose strategies that reduce operating costs. The SuperPro Designer computational tool is one of the more complete and recognized process design software packages. Also, it is a very versatile simulator that can meet the needs of engineers in a variety of industries, such as Biotechnology, Pharmaceutical, Chemical, Food, Mining, Wastewater Treatment, Control environmental, etc. That is why the purpose of this research is to evaluate the production of EPS from *Ganoderma lucidum* in large-scale suspension culture from a computational approach. The latter, by performing simulations using SuperPro Designer software to identify process yields and propose improvements aimed at increasing productivity. In this work, the software is calibrated with experimental data reported in the literature and based on the information found; strategies were proposed to identify the economic viability of the process.

2. Methodology

The development of this work was carried out through the following phases: (a) Definition of the culture medium for EPS production and *Ganoderma lucidum* growth. (b) Unit Operation Design for upstream and downstream processing according to Figure 1. (c) Simulation in SuperPro Designer software and analysis of results obtained in the first part. (d) Study of EPS production volume effect on operating costs.

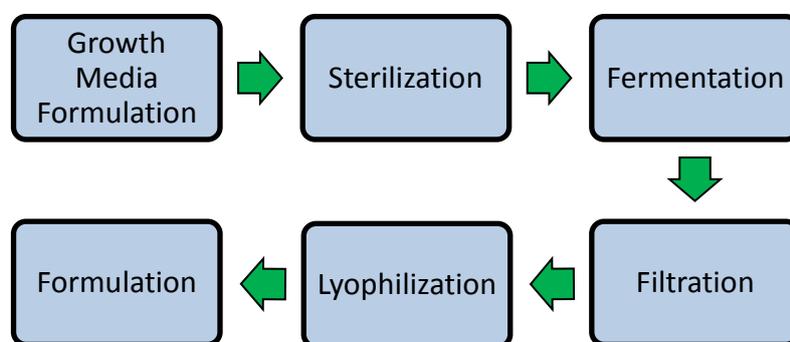


Figure 1. Unit operation Design for *Ganoderma lucidum* production.

2.1 Culture Media composition

The culture media used in the simulations is defined by the following composition [9] based on one liter: Glucose 35 g; Peptone 7.5 g and Yeast extract 2.5 g.

2.2 Unit Operations

The industrial production of a fungus through biotechnology can be carried out through the implementation of Up-stream operations as shown in Figure 1 (raw material processing, inoculum preparation, fermentation, etc.) and Down-stream (product separation and purification).

2.2.1 Raw Material Preparation. For obtaining the fermentation culture medium, the installation of an industrial tank with mechanical stirring is proposed [10-11]. The energy consumption by stirring at this stage is calculated by the equation (1):

$$P = N_p \rho N_i^3 d_i^5 \quad (1)$$

In which N_p is the power number with a value of 5.0, ρ is the culture media density 1000 kg / m³, N_i is the stirring speed 400 rpm and d_i is the impeller diameter.

2.2.2 Sterilization. Once the culture medium has been prepared, all external contaminants must be removed. In this work, heat sterilization is used, so that the growth medium for *Ganoderma lucidum* culturing does not have any thermo-sensitive components.

2.2.3 Growth of Ganoderma in Bioreactors. For growth on an industrial scale of *Ganoderma lucidum*, an inoculation train composed of three reactors is proposed in this research. Each one can supply 10% of inoculum. Based on the bibliographic references found, the operating parameters were adjusted to the required conditions: Stirring Speed: 500 rpm. The temperature fermenters were defined at 30 °C and the airflow at 0.5 vvm (air to reactor volume per minute) [1]. The stoichiometric reaction [12-13] for *Ganoderma* growth was defined in equation (2) as:

$$-\sum_{i=1}^N y_i m_i + \sum_{j=1}^M y_j m_j = 0 \quad (2)$$

Where y_i is the stoichiometric yield of each substrate i according to each component of the culture medium m_i (glucose, peptone, yeast extract and oxygen). The yield y_j refers to the stoichiometric coefficient of each product j (*Ganoderma* Biomass, exo-polysaccharides, CO₂ and H₂O). In this work, the biomass yield from the substrate was defined with a value of 0.10 g/g and the yield of EPS for simulations was programmed with a level of 0.038 g/g [9]. The biological process gives off heat to the environment so that it is characterized by being exothermic. It must be removed from the bioreactor so that the operating temperature remains constant. For the above, the heat \dot{Q} generated must be calculated to determine the cooling requirements. For this purpose, equation (3) is used:

$$\dot{Q} = -\Delta H_{rxn} - \dot{m}_w \lambda_w + P_0 \quad (3)$$

Here ΔH_{rxn} is the heat associated with the biochemical reaction, \dot{m}_w is the mass flow evaporated water, λ_w is the latent heat of vaporization and P_0 is the aerated power consumption generated by the stirring. It is calculated by equation (1) described previously.

2.2.4 Filtration. The biomass separation from culture media is carried out by a rotating drum filtration. There enters a feed stream from the production fermenter. The biomass and EPS are retained in the rotating drum and subsequently washed with a stream of water. In the latter procedure, traces of culture medium and other impurities from the fermentation process are removed.

2.2.5 Lyophilisation. Once the *Ganoderma* biomass and EPS filtration process have been carried out, the product must be dried to preserve its viability. That is why lyophilisation equipment is proposed at this stage. The volatile component is sublimated using vacuum pressure. It is worth mentioning that a freezing process of the biological material must be carried out before sublimation.

2.3 Performing the Simulation in SuperPro Designer Software

The development of simulations in SuperPro software was carried out based on the parameters mentioned above. The response variable that was pointed out in this work was the simulated operating cost (USD/g) according to the experimental data and settings used and it is calculated using the equation (4):

$$USD/g = \frac{\sum_{i=1}^N C_i}{Q_x V_R t_{oper}} \quad (4)$$

In this equation, C_i is the cost associated with each factor i (Raw materials, Energy, Discharges, Personnel, etc.). Q_x is the volumetric productivity. V_R is the volume of production and t_{oper} is the time in hours that the operation remains in operation plant for a year.

2.4 Evaluation of the effect of the volume and EPS yield on production costs

This research proposes a 3^2 factorial design with two factors to evaluate: Production Volume and EPS yield at three levels each (2; 20 and 200 m³ and 0.038; 0.059 and 0.072 g/g) to identify their effects on production costs and overall productivity. EPS yield values were taken from different bibliographic references that include the potentization of *Ganoderma lucidum* lines [14-15] with genetic engineering techniques to increase yields.

3. Results and Discussions

The main objective of this research was to evaluate the overall productivity of obtaining the *Ganoderma lucidum* fungus by submerged fermentation industrial scale from a computational approach using SuperPro Designer simulation software. In Consideration, operating costs were taken into account to identify the viability of the process. The operating conditions, the culture medium and the yields of biomass and exo-polysaccharides were taken from different bibliographical references as a starting point for the execution of the simulations. Figure 2 shows the simulated process diagram in this work in which it starts from a culturing media flow of 210 kg/h. At this stage, all components are mixed at a speed of 500 rpm in a storage tank (Storage). According to the results found, 3 KWh of energy is required to carry out this first stage of production. For the contaminant removal from the environment, continuous sterilization equipment (ST-101) is proposed, as previously explained. According to the simulated results, a steam flow of 12.16 kg/h is required to reach a holding temperature of 150°C for assuring the elimination of any contaminating agent. In this same stage, once the sterilization process is finished, the culture medium is cooled to fermentation temperature of 30 °C. To achieve the above, the results suggest a cooling water flow of 3880.5 kg/h at an inlet temperature of 25 °C. Upon completion of the cooling process described above, the sterile culture medium must be pumped to the seed (SFR) and production fermenters (FR), respectively. In the latter, *Ganoderma* fungus grows and generates the required exo-polysaccharides according to the yields reported in the bibliographic references mentioned previously.

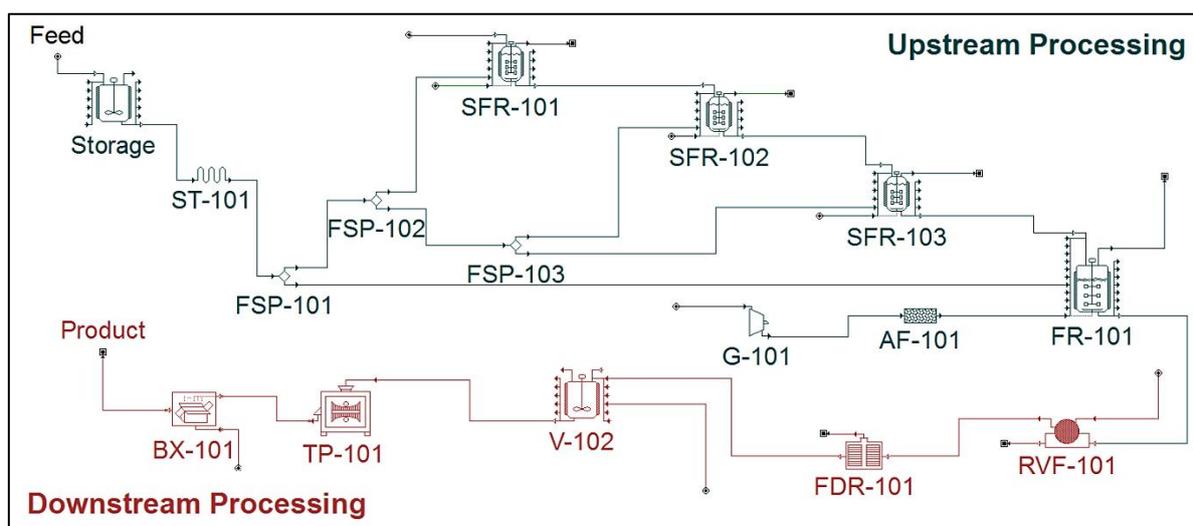


Figure 2. Large scale *Ganoderma lucidum* production proposed for the simulations.

It should be mentioned that microbial growth is characterized by being an exothermic process that gives off heat to the medium, therefore the excess of it being extracted in a controlled manner to maintain the optimum fermentation temperature. According to the results found, fermenters can generate 4.2057 kWh (production bioreactor) of exothermic heat generated during the fermentation process. According to the results found, a flow of 1423.31 kg/h of cooling water at a temperature of 5 °C is required to extract excess heat and thus maintain the temperature in the fermentation equipment. Likewise, the growth *Ganoderma* is an aerobic process; therefore, it involves oxygenation. For this purpose, this research proposes the use of an air compressor (G-101), and according to the results obtained, this equipment must be designed to generate an airflow of 18164.93 kg/h with an energy consumption of 21.94 kW.

At this same stage, the output streams predict a glucose concentration of 7.48 g/l, which indicates that the substrate conversion is 70% in such a way that the depleted culture medium still contains trace components that must be removed. Future research is required at this stage to reuse the mentioned micronutrients. Even so, the biomass and extracellular exo-polysaccharides must be removed from the depleted culture medium. That is why in this phase of the process, a rotary drum filtration (RVF-101) is proposed that removes biomass and EPS of the spent fermentation medium. According to the calculations found in this equipment, 0.67 kg/h of biomass, 0.27 kg/h of EPS enter to be concentrated in a filter cake, where the biological material is then washed to remove impurities from the culture medium. According to the simulation results, a rotating drum filter with a filtration area of 0.08 m² and a flow of 206.235 kg/h is required for this stage to be successful. Based on the above and to preserve the viability of the biological material, the *Ganoderma* biomass and EPS must be dried. One of the most traditional unit operations to dry biological material is lyophilisation. The latter consists of a sublimation process: water is removed at a vacuum pressure for keeping the biological activity of *Ganoderma*. According to the results found, freeze-drying equipment (FDV-101) with a sublimation capacity of 1.44 kg and a drying area of 0.15 m² is required. At this stage, the solid phase product is discharged at a flow of 0.95 kg/h. In the large-scale *Ganoderma* formulation stage, the product must be mixed with adjuncts or molecules that stabilize and preserve its biological activity. Based on the latter, the use of hypromellose is proposed in this research. For the above, according to the simulation data obtained, a 1-liter tank (V-102) is required to discharge the product in bulk at a flow of 0.21 L/h and energy consumption of 2.6 kW. Finally, it is proposed in this investigation that the product be formulated in the form of pills. Implementation of this procedure, a pelletizer (TP-101) is used to generate a flow of 2154 tablets/h.

According to the results reported by simulation with the SuperPro Designer software, in the 2 m³ plant, the overall productivity of 0.237 kg/h of biomass and exo-polysaccharides is calculated. Therefore, each gram of product has an operating cost of 6.82 USD. In contrast, according to

bibliographic reports, the value of *Ganoderma* in the world market can range around USD 0.50/g [8]. According to the findings obtained in this research, the operating cost for producing *Ganoderma* by submerged cultivation at a production level of 2 m³ is much higher than that reported in the references mentioned. The above suggests the need for the implementation of strategies that allow identifying the parameters required to reduce the operating costs or increase the overall productivity that will enable the competitiveness of obtaining *Ganoderma* through controlled cultivation strategies submerged in bioreactors. The above explained in equation (4), which numerically determines the production costs associated with *Ganoderma*. According to the mathematical model mentioned, the unit cost of the product can be reduced in two ways: the first option may be to reduce the total operating expenses (materials, energy, personnel, etc.), and the second way is based on increasing the overall productivity of the process. The latter was taken into account in this research to perform a sensitivity analysis and identify different critical parameters in the large-scale operation. So that the increase in production volume was raised to a final value of 200 m³. Simultaneously, the large-scale process was evaluated with different exo-polysaccharide yields reported in the literature based on the *Ganoderma lucidum* strain that has been genetically improved to achieve yields of up to 0.072 g/g. These parameters are presented in Figures 3-4.

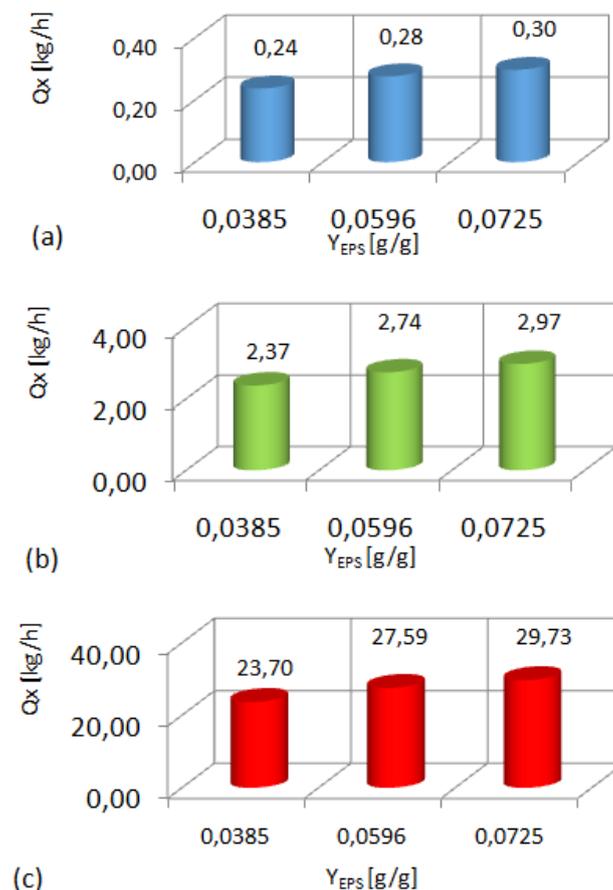


Figure 3. Q x Productivity vs EPS yield Y_{EPS}. (a) Volume 2 m³. (b) Volume 20 m³ y (c) Volume 20 m³.

According to the results obtained (Figure 3) (especially for cases at volume kept constant), the increase in productivity achieved is almost negligible in comparison with the productions performed by the genetically modified strain.

Operating costs can only be reduced by 15%, calculating values of approximately USD 5/g in a 2 m³ bioreactor and an exo-polysaccharide yield of 0.072 g/g (see yellow region Figure 4). In contrast,

according to simulations performed at different operating volumes, the cost per gram of product is significantly reduced, with values of up to 0.17 USD/g being found in a 200 m³ bioreactor (see dark blue region Figure 4) using the original strain (not genetically modified). Similar values (0.14 USD/g) were found using the same production volume (200 m³) and the genetically modified strain, which suggests that the factor that most influences unit product costs are the size of the production bioreactor. Based on the above, to achieve values similar to those reported in the literature and with a 50% utility, an average production volume of 80 m³ is required for the technique of obtaining *Ganoderma* by submerged large-scale cultivation to be viable.

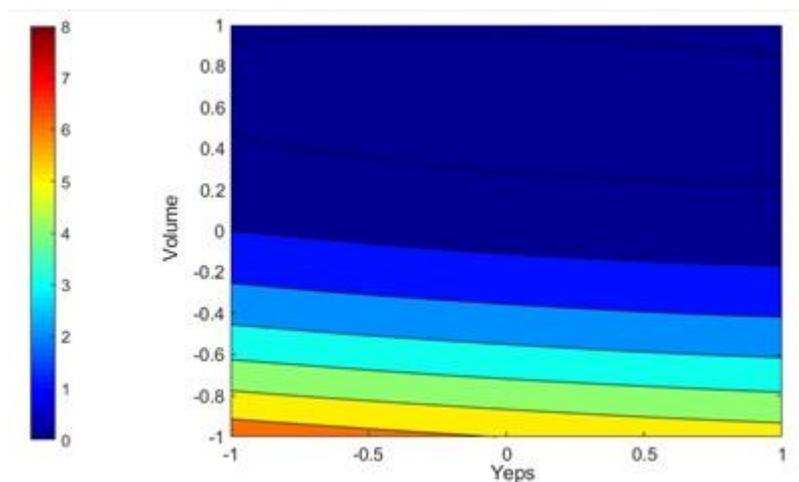


Figure 4. Contours of volume and EPS yield on operating costs for large scale *Ganoderma lucidum*.

The total operating costs in an industrial scale process are discriminated according to the consumption of raw materials, personnel, process monitoring for quality control, waste disposal and costs of services required or utilities (energy, heating steam, cooling water, etc.). The results obtained for each parameter evaluated in this work are observed in Figure 5. Results once again support the significant effect of *Ganoderma* production volume in unit costs. In such a way that the fraction of the cost associated with personnel is reduced from an average value of 48% (2 m³) to a level of 19 % (200 m³) in the evaluated cases. The above could be explained, taking into account that the submerged cultivation of *Ganoderma* in a mostly automated technology, therefore, the required amount of labour contracted to operate a 2 m³ production plant, does not significantly depart from the needs of operation in a 200 m³ plant production.

Contrary to the above, the percentage of costs associated with raw materials and equipment use, begin to have importance under the volume effect (Figure 5). The latter considering also the increase in reagents requirements, culture media, equipment maintenance, insurance policies, local taxes, and depreciation costs. According to the results shown in this research, a large scale culture technology submerged in bioreactors would be a very productive process at operating volumes more significant than 80 m³. The previous would assure a competitive price in the market. However, scale-up studies are required to verify the productivity of bioreactors (80-200 m³).

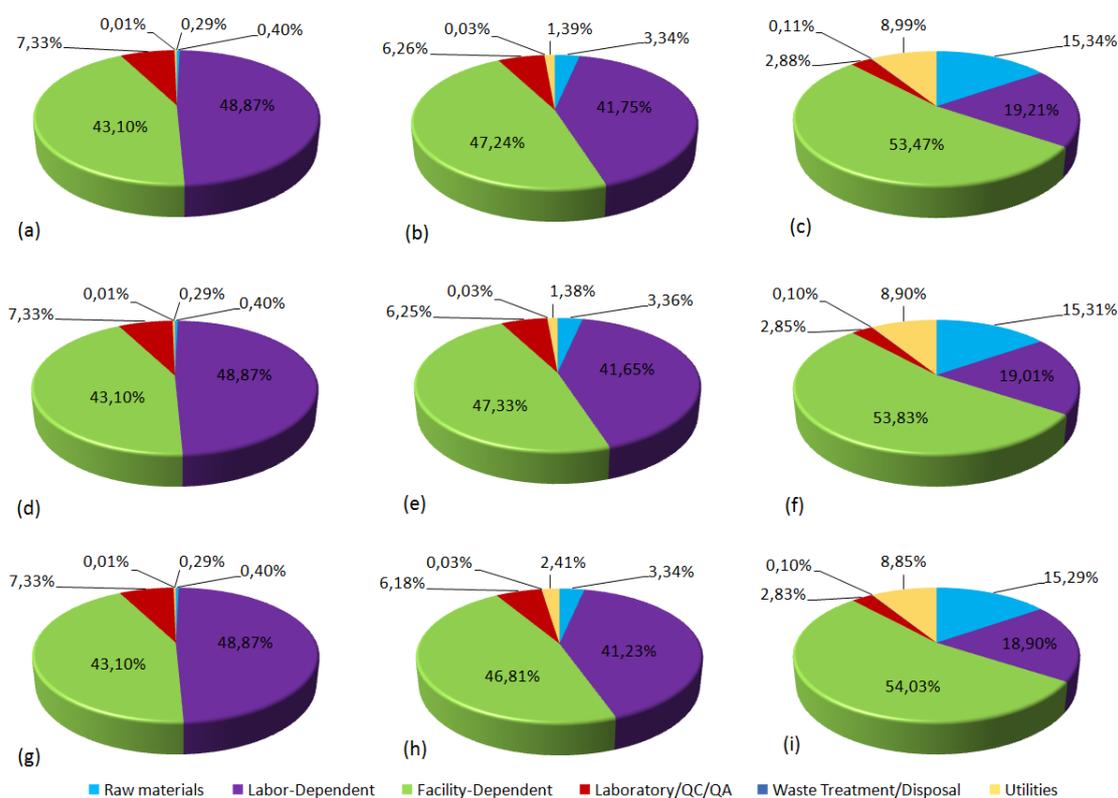


Figure 5. Effect of EPS yield on operating costs for large scale *Ganoderma lucidum*. On rows (F_i), effect of EPS yield for the same volume it is shown ($F_1: 2; F_2: 20$ y $F_3: 200$ m³) and columns (C_i), volume impact to EPS yields is observed ($C_1: 0,038, C_2: 0,059$ y $C_3: 0,072$ g/g).

4. Conclusions

The bioreactor volume significantly affects production costs compared to the exopolysaccharide yields evaluated. Values of USD 6.82/g in a 2 m³ bioreactor are estimated while costs are reduced considerably to 0.8 USD/g in a 20 m³ production volume. The findings found here demonstrate the importance of predicting a large-scale bioprocess focused on improving the overall productivity of a biotechnological product. EPS yield has no significant effect on cost reduction. An average production volume of 80 m³ is required to reach similar viable values to those reported in the literature.

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