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Economic Assessment of Itaconic Acid Production from Aspergillus Terreus using Superpro Designer

To cite this article: L Nieto *et al* 2020 *J. Phys.: Conf. Ser.* **1655** 012100

Economic Assessment of Itaconic Acid Production from Aspergillus Terreus using Superpro Designer

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Abstract. Itaconic acid is a metabolite produced from biotechnological pathway using the microorganism *Aspergillus terreus*. It is used in a wide number of sectors such as industry, agriculture and health. Based on its chemical properties, itaconic acid is a great interest for replacing polymers and resins derived from petroleum. However, its production is still expensive and this latter hinders large scale studies regarding itaconic acid production. Nowadays, simulation is a great alternative to overcome this problem. It allows researchers to make a more effective evaluation and self-assessment, reducing costs and avoiding losses at large scale operation. For that reason, the purpose of this research is performing an Itaconic acid production simulation on industrial scale using SuperPro Designer software. Costs reduction improvement is also analyzed. 90g/L of glycerol, 1g/L of NH_4NO_3 and 1.25 of KH_2PO4 are set up on simulation as media composition reported from references. Results regarding itaconic acid crystals showed a productivity of 171 kg/h and an operating cost closed to 42.0 USD/kg. Interestingly, a 17 % productivity increase is reached by proposing a stream recycling based on itaconic acid recovering from downstream centrifugation processing. The latter based on a higher productivity estimated (200 kg/h). Also average costs are reduced at 12 % since 38.1 USD/kg is found using improvements mentioned. Results found here demonstrate the potential usage of simulators for estimating costs and production which allows predicting the bioprocess feasibility.

1. Introduction

Itaconic acid (IA) is metabolite donating 2 H+ protons per molecule in aqueous solution. It is composed of five carbons and two carboxyl groups. Itaconic acid is also known as methylene succinic acid and is used in a wide range of sectors such as industry, agriculture and health [1]. IA has certain characteristics conferring a great interest in the world market. Therefore, Itaconic acid has been well studied regarding production methods and polymerization. Its structural similarity compared to several acrylic derivatives has generated some interest in the polymer industry [2-4] That is why IA is used to produce mostly hydrogels, polyesters, plastics, artificial glass, paints, textiles and glues. IA is also used in medicine, cosmetics, lubricants, herbicides, wool modifiers and in the detergents manufacture, water treatments, dispersants and adhesives [5] Currently, Acid itaconic denominates 1 of the 12 biotechnological products used as precursor in the production of various high-value chemicals by the U.S. Department of Energy [2-4]. IA is produced by sugar fermentative a pathway using the microorganism *Aspergillus terreus* [5]. The latter is a thermo-tolerant fungus with optimal growth at 35-40 ºC. Itaconic acid is an important chemical platform that has a wide range of potential and real applications. It can be used to replace a wide range of petroleum-based chemicals such as acrylic and methacrylic acids. It will reduce dependence on oil and harmful environmental effects [6].

However, it only occupies a niche market due to its high cost relative to acrylic acid and other alternatives, which limits their use to low-volume markets. Today the largest producer of itaconic acid is China, exporting almost 78% of the world's itaconic acid. Likewise, the South of America is a great import of itaconic acid. Based on the latter, design and construction of a large scale itaconic production plant is great of interest for south American countries. Even so, exports have been led by China, India, united states, Japan, South Africa and United Kingdom [7] Global production is estimated to be 80000 tons per year at around price and production is expected to grow by 5.5% each year between 2016 and 2023 [6].

Regarding large scale IA technologies, information related to IA industrial production is scarce. Its operating costs are expensive and makes research difficult [8-9]. Nowadays, new technologies and advances in computing have been developed for enhancing new researches. One of these computer-aided tools is the bioprocess simulation, which allows to obtain a variety of results and even, it analyse multiple solutions for determining a process profitability. Simulation is a great advantage for researchers to make a more effective assessment by identifying gaps and proposing possible process optimizations for reducing operating costs [10]. Based on the latter, motivation of this paper is proposing an itaconic acid industrial process from *Aspergillus terreus*. Simulations are performed using SuperPro Designer software and especial emphasis was focused on productivity and operating cost improvements.

2. Methodology

2.1. Upstream processing

For performing simulations in SuperPro Designer software, culture media conditions, upstream and downstream unit operations for IA large scale production are required (see Figure 1). Simulation setup is performed based on experimental data and operating conditions collected from references.

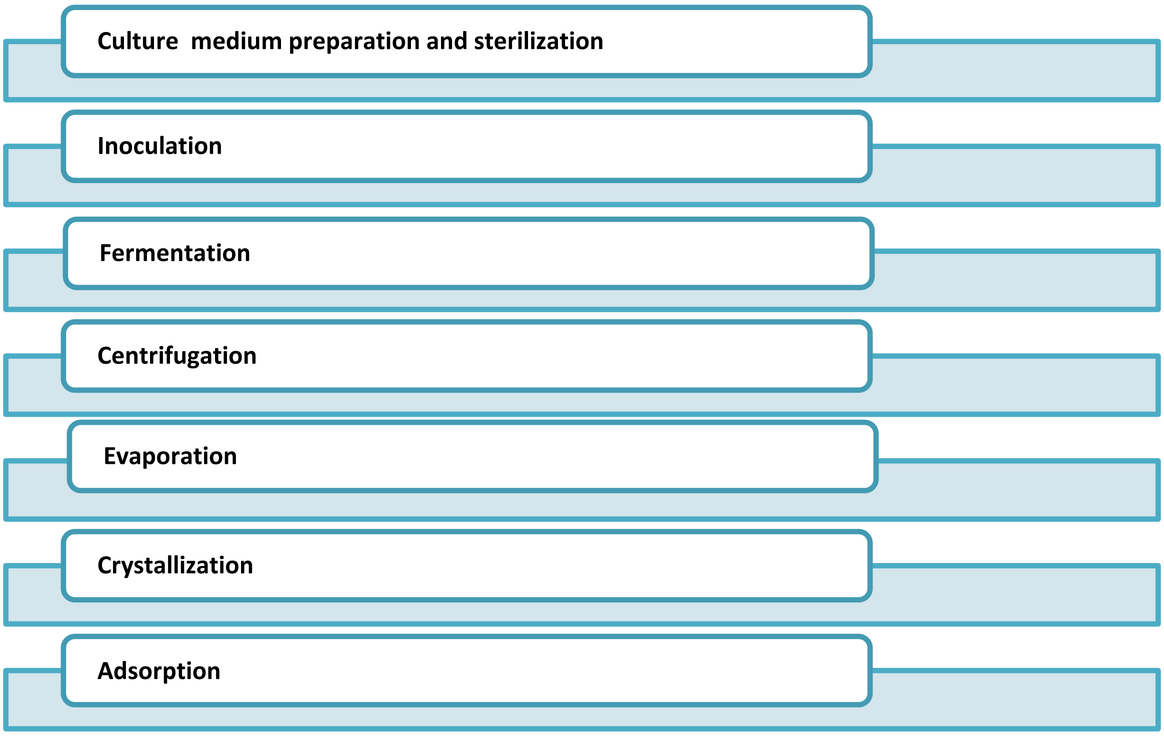

Figure 1. Unit operation for Itaconic Acid Production.

2.1.1. Culture Media preparation. The culture medium used in simulations was reported from the literature [11] and is defined by the following composition: 90 g/L of glycerol, 1 g/L of NH_4NO_3, 1.25 g/L of KH_2PO_4. The culturing media flow was set at 5000 kg/h in in four mixing tanks. The power input at mixing tank was set up at 3 kW/m^3. Subsequently, this medium was sterilized with a sterilizer with a sterilization holding temperature of 140°C and a specific death rate of 2.44 s^{-1}. 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.

2.1.2. Inoculum Preparation and Fermentation. Aspergillus terreus is pre-cultured at 37 ºC using a seed fermenter train composed of 5 bioreactors. The first reactor is fed with a volume of 0.07 m3, the second one with 0.7 m3, the third one at 7.0 m^3 and last two inoculum tanks are filled with 40.0 m^3 each. Finally, 3 production fermenters are proposed using a carrying capacity of 240.0 m^3 (each tank). Based on the literature [11], the operating conditions of the fermenters were: 37° C, power input of 3 kW/m3and an air flow of 2 vvm (air to reactor volume per minute). Continuous fermentation is established. The sterile nutrient solution is continuously added to the bioreactors and an equivalent amount consumed for the microorganisms is removed simultaneously from the system. The biomass Yxs and the product yield Yps from glycerol are set up as 0.27 and 0.44 g/g, respectively [11]. Fermentation time used for SuperPro Designer simulations is defined at 6 days [11-12]. 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). The overall reaction equation in each fermenter is modelled according to equation (1):

$$Glycerol + O_2 \rightarrow Biomass + Itaconic\ Acid + CO_2 + H_2O \quad (1)$$

2.2. Downstream Processing
For IA recovering, a down-stream processing is performed in SuperPro Designer software based on references. Unit operations and operating conditions are set up based on references [12-14].

2.2.1 Separation. In order to remove biomass produced from previous fermenters, down-stream processing was carried out, starting with a centrifugation unit operation. Liquid stream resulted from centrifugation is collected for further treatment. A biomass removal using a Disk Stack type centrifuge is proposed based on literature [13].

2.2.2. Evaporation. For removing water to increase itaconic acid concentration two multi-effect evaporator were used for reaching a concentration of 350 g/L regarding evaporation unit operations [14]. Evaporation economy is estimated at 0.82 kg vapor/kg heating agent.

2.2.3. Crystallization. Crystallization is a classical method of IA recovery produced by fermentation process [12-14]. Based on references, itaconic acid is easily recovery by cooling at 15°C. For simulation set up, two continuous crystallizers with a crystallization yield of 80 % [14] were proposed. First one with a working volume of 1089.16 L and second one with 390.12 L. Heating was referred as water component with a heating temperature of 100 °C for both crystallization units, resulting in a heating duty of 162637 and 11484 kcal/h, respectively. As mentioned before, cooling temperature was set up at 15 °C for both cases using chilled water at 5 °C.

2.2.4 Impurities Removal. Crystals formed need to decolored for removing impurities. In this research a granular activated Carbon adsorption procedure is proposed for impurities removal. This unit operation simulates the performance of a packed bed adsorption column. Granular activated carbon is used as default adsorbent. The target purity is set up at 95 % [2].

2.3 Cost Estimation
Costs are calculated as total capital investment and operating costs [10]. Total Capital Investment refers to the fixed costs that are associated with a process. This is calculated as the sum of the following cost items over all sections of a process: 1. Direct Fixed Capital (fixed assets of an investment, such as plant and equipment); 2. Working Capital (tied-up funds required to operate the

business); 3. Startup and Validation Cost (pre-opening, one-time expenditures incurred to prepare a new plant for operation); 4. Up-Front R&D Cost (research and development required before a product is manufactured); 5 Up-Front Royalties (payments made for use of assets, resources, patents, etc). The operating cost of a project includes costs that are related to the demand for a number of resources (i.e., raw materials, consumables, labor, heating/cooling utilities and power), as well as additional operational costs. More specifically, the annual operating cost (AOC) is calculated as the sum of the following kind of cost: 1. Materials cost; 2. Consumables cost; 3. Labor-dependent cost; 4. Utilities (heating/cooling utilities and power) cost; 5. Waste treatment/disposal cost; 6. Facility-dependent cost (equipment maintenance, depreciation of the fixed capital cost, and miscellaneous costs such as insurance, local (property) taxes and possibly other overhead-type of factory expenses); 7. Laboratory/QC/QA cost; 8. Transportation cost; 9. Miscellaneous costs (on going R&D, process validation, oher overhead-type expenses not covered by other categories); 10. Advertising/selling costs; 11. Running royalties; 12. Failed product disposal cost. Finally, IA unit operating cost ($^{USD}/_{g}$) is calculated using the equation (2):

$$^{USD}/_{g} = \frac{\sum_{i=1}^{N} C_i}{Q_p V_R t_{oper}} \quad (2)$$

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

3. Results and Discussions

The goal for this work is to perform a computer-aided estimation of Itaconic acid production on industrial scale from *Aspergillus terreus.* Simulations are set up using SuperPro Designer software and especial emphasis was focused on productivity and operating cost improvements. Operating were referred from bibliography based on experimental and theoretical findings [2, 11]. Figure 2 shows the unit operations proposed for large scale simulation of itaconic acid.

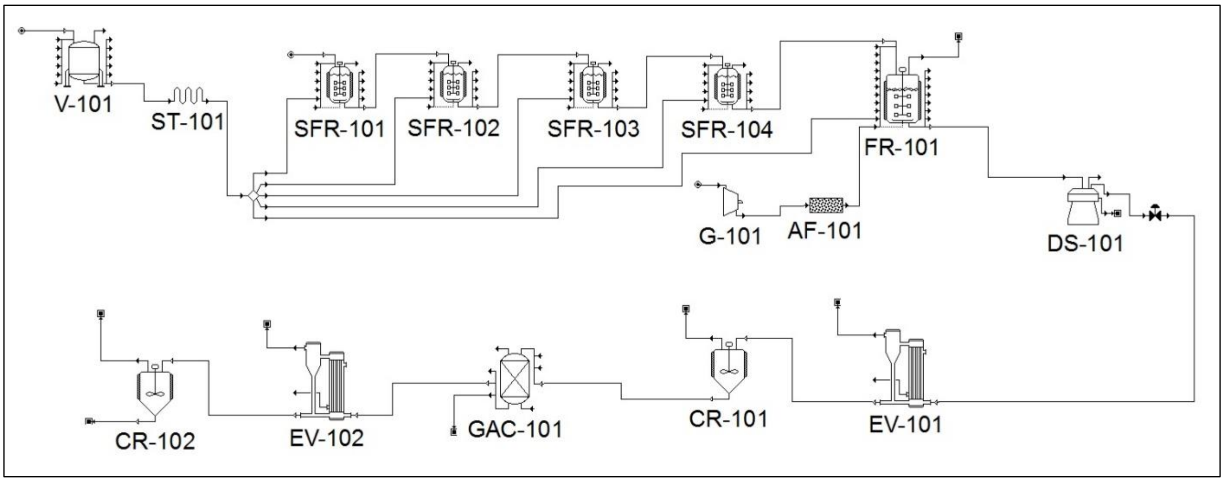

Figure 2. Large Scale production of Itaconic acid simulation using SuperPro Designer Software.

As mentioned before culturing media is pumped at 5000 kg/h (see Table 1). At this stage, all components are mixed using four mixing tank (V-101). For contaminant removal a continuous sterilization equipment (ST-101) is placed before fermentation. According to the simulated results, a steam flow of 12.16 kg/h is required to reach a holding temperature of 140°C. Once sterilization process is finished, medium is cooled to fermentation temperature of 37 °C. To achieve the above, a cooling water flow of 3880.5 kg/h is estimated.

Table 1. In and Out streams estimated from SuperPro Designer at Media Preparation Zone.

Media Preparation		
Material	IN	OUT
Glycerol (kg/h)	450.00	450.00
KH_2PO_4 (kg/h)	6.25	6.25
NH_4NO_3 (kg/h)	5.00	5.00
Water (kg/h)	4538.75	4538.75
Temperature (ºC)	25.00	37.00
TOTAL	5000.00	5000.00

Based on equation 1, biomass is produced from glycerol carbon source. According to references, each gram of glycerol is used to form 0.27 g of biomass and 0.44 g of IA. Mass balance for stoichiometric biomass growth from Fermentation zone is reported on Table 2.

Table 2. In and Out streams estimated from SuperPro Designer at Fermentation Zone.

Fermentation		
Material	IN	OUT
Glycerol (kg/h)	450.00	40.91
IA (kg/h)	0.00	200.00
Biomass (kg/h)	0.00	122.73
KH2PO4 (kg/h)	6.25	0.00
NH4NO3 (kg/h)	5.00	0.00
CO2	0.00	32.71
Water (kg/h)	4538.75	4603.65
Temperature (ºC)	37.00	37.00
TOTAL	5000.00	5000.00

As shown in Table 2, 450 kg/h of glycerol is fed at fermentation zone using a pump (PM-101) to produce 200 kg/h itaconic acid (IA) and 122.73 kg/h of biomass resulted from the fermentative growth.

Fermentation zone is composed of 5 seed fermenters (3 reactors SFR (101, 102 and 103) and 2 reactors SFR-104), three large scale fermenters (FR-104), and air compressor (G-101) and an air filtration system (AF-101). Also, the exhausted carbon source medium can be observed.

The flow of this stream was calculated as 40.91 kg/h and a 91 % substrate consumption is reached. Even so, the biomass and extracellular IA need to be removed from the culture medium.

That is why next phase of the process consist of solid liquid removal unit operation and results are shown in Tables 3-4.

Table 3. In and Out streams estimated from SuperPro Designer at Centrifugation Zone.

Centrifugation			
Material	IN	OUT Solids	OUT Liquids
Glycerol (kg/h)	40.91	5.83	35.08
IA (kg/h)	200.00	28.49	171.51
Biomass (kg/h)	122.73	122.73	0.00
Water (kg/h)	4603.65	655.87	3947.78
Temperature (ºC)	37.00	44.00	44.00
TOTAL	5000.00	812.92	4154.37

Table 4. In and Out streams estimated from SuperPro Designer for evaporation, granular activated carbon and crystallization.

Evaporation I			
Material	IN	OUT Product	OUT Water
Glycerol (kg/h)	35.08	35.08	0.00
IA (kg/h)	171.51	171.51	0.00
Water (kg/h)	3947.78	275.89	3671.89
Temperature (ºC)	44.00	40.00	40.00
TOTAL	4154.37	482.48	3671.89
Crystallization I			
Material	IN	OUT Product	OUT Water
Glycerol (kg/h)	35.08	35.08	0.00
IA (kg/h)	171.51	34.30	0.00
IA Cristals (kg/h)	0.00	137.21	0.00
Water (kg/h)	275.89	27.59	248.30
Temperature (ºC)	44.00	15.00	100.00
TOTAL	482.48	234.18	248.30
GAC Column			
Material	IN	OUT Product	OUT waste
Glycerol (kg/h)	35.08	0.00	35.08
IA (kg/h)	34.30	34.30	0.00
IA Cristals (kg/h)	137.21	137.21	0.00
Water (kg/h)	14426.04	27.59	14398.45
Temperature (ºC)	15.00	15.00	25.00
TOTAL	14632.63	199.10	14433.53
Evaporation II			
Material	IN	OUT Product	OUT Water
IA (kg/h)	34.30	34.30	0.00
IA Cristals (kg/h)	137.21	137.21	0.00
Water (kg/h)	27.59	2.16	25.43
Temperature (ºC)	15.00	40.00	40.00
TOTAL	199.10	173.67	25.43
Crystallization II			
Material	IN	OUT Product	OUT Water
IA (kg/h)	34.30	6.86	0.00
IA Cristals (kg/h)	137.21	164.65	0.00
Water (kg/h)	2.16	0.22	1.94
Temperature (ºC)	40.00	15.00	100.00
TOTAL	173.67	171.73	1.94

For biomass removal, a disk stack (DS-101) is proposed based on references [13]. Therefore, a solid out stream with 122.73 kg/h of biomass is removed from the culture media. The foregoing constitutes the starting point for possible improvements focused on losses recovery. These developments will be discussed later. Liquid stream resulted from centrifugation contains 171.51 kg/h of itaconic acid and it is further treated for recovering using evaporation, granular activated carbon and crystallization downstream processing (see Table 4). The purpose of the unit evaporation operation, as mentioned above, is to concentrate the final product by removing water (see Figure 2). In such a way, desirable concentration at equipment exit (EV-101) was set up with a value of 350 g/L. According to the results obtained (see Table 4), first evaporation equipment requires a water removal capacity of 3,671.89 kg/h for targeting this objective. Once the product is concentrated, a crystallization step (CR-101) is necessary for itaconic acid recovering (crystals form). According to bibliographic reports, it is possible to obtain a crystal conversion efficiency of 80% [2].

Based on the above, the results suggest a crystallizer with a capacity of 1089 liters and a cooling capacity of 15 ºC. At this same stage, water is also removed with an efficiency of 90%. However, once crystallization been completed, itaconic acid still contains impurities or residues of culture media that must be removed before its final concentration. Based on the above, it is proposed in this investigation (see Figure 2) the use of an activated carbon column packed (GAC-101). The mentioned component is characterized by having a large amount of micro-pores. In such a way that these micro-pores provide conditions for the impurity adsorption process to arise. According to the results obtained by simulation in SuperPro Designer, an impurity removal zone is required, equipped with 25 columns, each with a loading capacity of 27 m^3. Finally, with the aim of concentrating the itaconic acid to a purity level of 95%, product is treated again by means of additional evaporation units (EV-102) and crystallization (CR-102) to achieve a standard productivity of 171.73 kg/h. Based on the latter, the large scale IA plant is designed to reach a productivity of 1360 t/year with 95% purity.

The total operating costs in an IA 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.). Figure 3 shows the operating cost distribution for itaconic acid production.

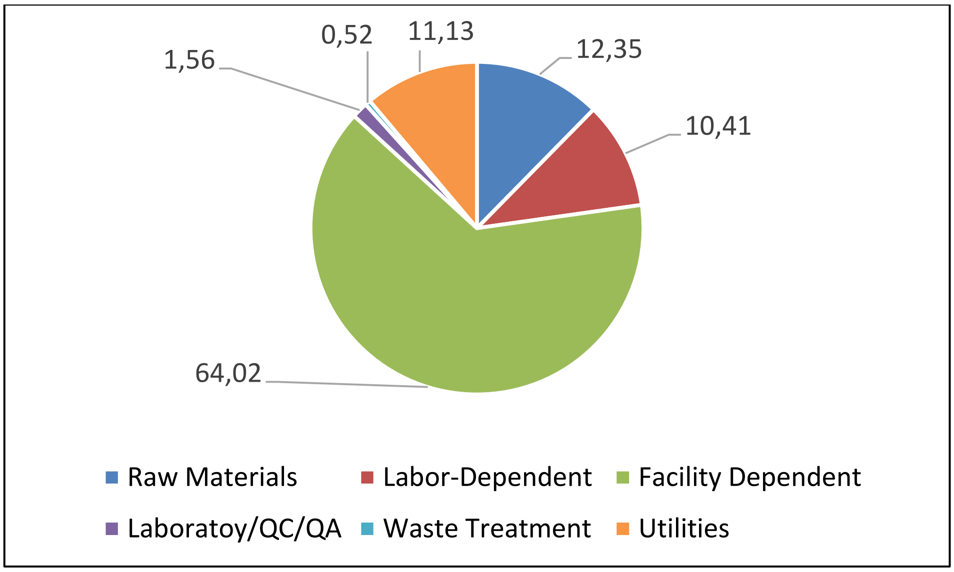

Figure 3. Operating costs per year (standard process).

According to calculations, the production cost per kg is 42.69 USD. Based on Figure 3, facility dependent costs represent significant operating cost due to its influence on 64 % regarding all operating costs for producing IA at a large scale. Better appreciation of operating costs estimation related to each unit operation zone is shown in Figure 4.

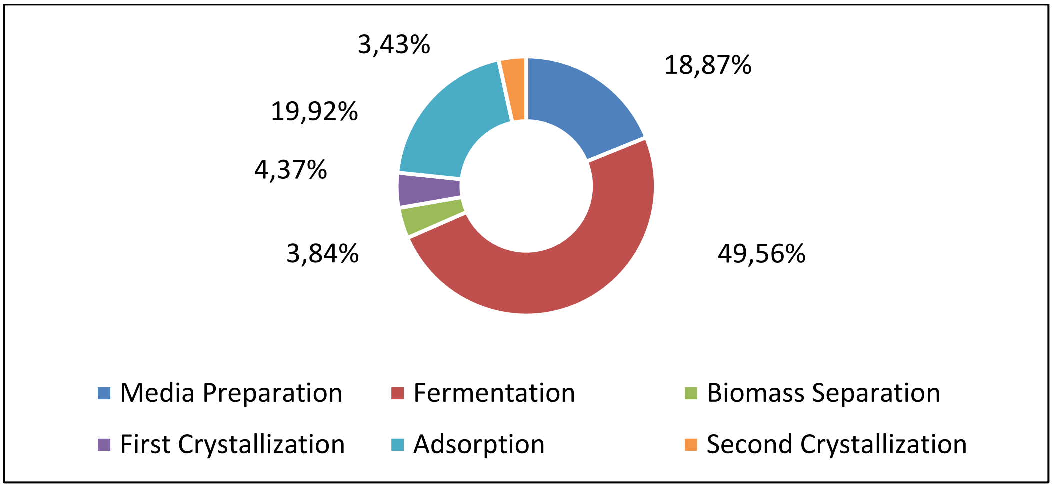

Figure 4. Operating costs per year according to each unit operation zone (standard process).

As shown in Figure 4, 49.56% of the costs are associated with the large-scale fermentation process. This is due to the large requirement at large equipment that significantly influences costs related to facilities (use of equipment and its maintenance) [10]. Currently, it is possible to obtain AI in a commercial value in the range between 46 USD/kg from the supplier Sigma-Aldrich Germany. This means that improvements must be considered in the production plant. As previously explained in Table 3, product losses associated with waste discharge stream are generated during the centrifugation process, which are equivalent to 28.49 kg/h of itaconic acid. Taking this into account, approximately 230 tons of product are lost during one operation year, which can significantly affect the viability of the operation plant. That is why, it is proposed in this investigation the addition of a filtration system (RVF-101) in order to recover the product at the output stream of the centrifuge (DS-101) as seen in Figure 5.

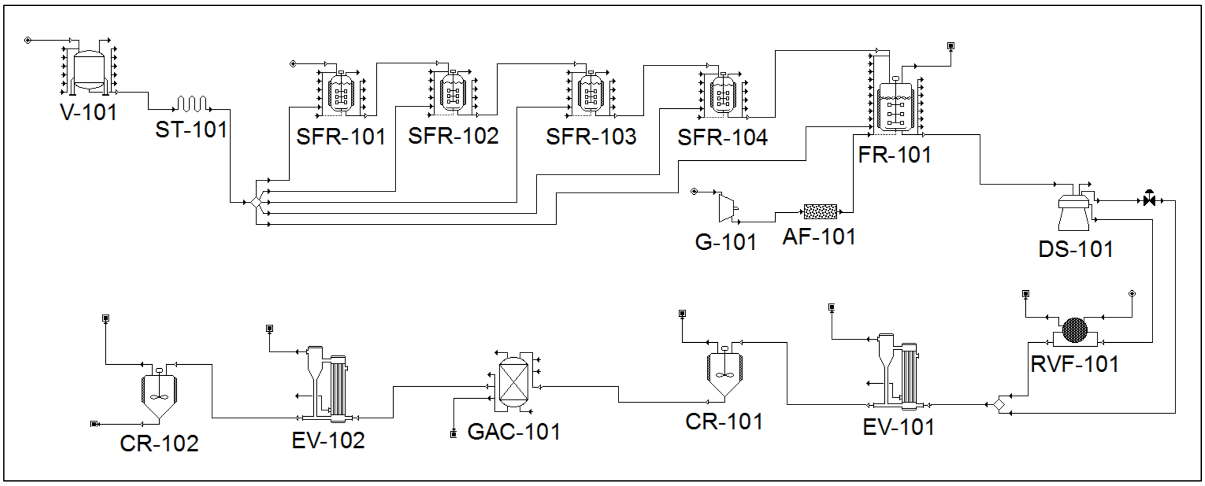

Figure 5: Improved Large Scale production of Itaconic acid simulation

Tables 5-6 present the results of the proposed improvement.

Table 5. In and Out streams in Filtration Zone (improved).

Filtration			
Material	IN	OUT Solids	OUT Liquids
Glycerol (kg/h)	5.83	0	5.83
IA (kg/h)	28.49	0	28.49
Biomass (kg/h)	122.73	122.73	0.00
Water (kg/h)	755.87	82.39	673.89
Temperature (ºC)	37.00	36.2	43.30
TOTAL	5000.00	812.92	4154.37

Table 6: In and Out streams for evaporation, granular activated carbon and crystallization downstream processing(improved).

Evaporation			
Material	IN	OUT Product	OUT Water
Glycerol (kg/h)	40.91	40.91	0.00
IA (kg/h)	200.00	200.00	0.00
Water (kg/h)	4621.27	321.83	4299.44
Temperature (ºC)	44.00	40.00	40.00
TOTAL	4862.18	526.74	4299.44
Crystallization I			
Material	IN	OUT Product	OUT Water
Glycerol (kg/h)	40.91	40.91	0.00
IA (kg/h)	200.00	40.00	0.00
IA Crystals (kg/h)	0,00	160.00	0.00
Water (kg/h)	321.83	32.18	289.65
Temperature (ºC)	40.00	15.00	100.00
TOTAL	562.74	273.09	289.65
GAC Column			
Material	IN	OUT Product	OUT waste
Glycerol (kg/h)	40.91	0,00	40.41
IA (kg/h)	40.00	40.00	0,00
IA Crystals (kg/h)	160.00	160.00	0,00
Water (kg/h)	16822.75	32.18	16790.57
Temperature (ºC)	15.00	15.00	25.00
TOTAL	17063.66	232.18	16830.98
Evaporation II			
Material	IN	OUT Product	OUT Water
IA (kg/h)	40.00	40.00	0,00
IA Crystals (kg/h)	160.00	160.00	0.00
Water (kg/h)	32.18	2.52	29.67
Temperature (ºC)	15.00	40.00	40.00
TOTAL	232.18	202.52	29.67
Crystallization II			
Material	IN	OUT Product	OUT Water
IA (kg/h)	40.00	8.00	0.00
IA Crystals (kg/h)	160.00	192	0.00
Water (kg/h)	2.52	0.25	2.26
Temperature (ºC)	40.00	15.00	100.00
TOTAL	202.52	200.25	2.26

According to the results obtained with the proposed improvements, an AI productivity of 200 kg/h is achieved, suggesting an increase of 17% (see Figure 6). Also average costs are reduced at 12 % since 38.1 USD/kg is found using improvements mentioned. Results found here demonstrate the potential usage of simulators for estimating costs and production which allows predicting the bioprocess feasibility.

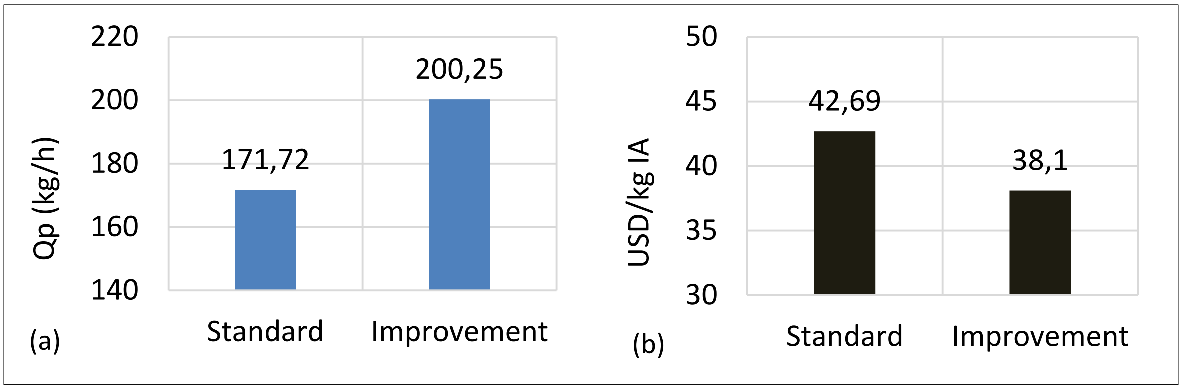

Figure 6. Improved Itaconic acid Productivity (a) and Total USD (b) calculated by SuperPro Designer.

The above improved unit operation cost is explained based on in equation (2), which numerically determines the production costs associated with IA. According to the mathematical expression 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 IA productivity. The latter was used in this research due to the stream losses identified at the standard large scale IA process for further recovering. So that, the increase in productivity raised to a final value of 200 kg/h.

4. Conclusions

The bioreactor volume significantly affects production costs compared to the exopolysaccharide yields evaluated. Values of USD 6.82/g in a 2 m^3 bioreactor are estimated while costs are reduced considerably to 0.8 USD/g in a 20 m^3 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 m^3 is required to reach similar viable values to those reported in the literature.

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