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Batch Process Modelling and Economic Evaluation of Glucuronidase Enzyme Production

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Abstract

β-glucuronidases enzyme is a valued product in the pharmaceutical industry. This work features the modeling and optimization of *β*-glucuronidases enzyme production from recombinant *E. coli* using SuperPro Designer v5, a commercial batch process simulator. The study focuses on designing an economically viable *β*- glucuronidases enzyme production process. In the base case process, an annual production of 956 batches (corresponding to 8,523 kg) of *β*- glucuronidases enzyme was made. Seven alternatives production schemes were further developed for increased production using batch debottlenecking strategy. The best alternative scheme was reported to achieve a product yield of 100% increment, with an annual production of 1912 batches of *β*- glucuronidases enzyme. Economic analysis determined that the proposed alternative scheme has an annual revenue of USD 44M, with a 15.24% gross margin and a 29.44% of return on investment. The payback period of this scheme was estimated to be less than four years.

Keywords: *β*- glucuronidases enzyme; Recombinant *E. coli*; Process simulation and optimization; Batch processes; Debottlenecking; Economic analysis

Introduction

β- glucuronidase is an enzyme responsible for the degradation of various polysaccrides or cleavage of glucurono-conjugates. It is commonly found in plants, insects, bacteria and animals (particularly high concentration in the liver). It catalyzes the hydrolysis of *β*-glucuronidase conjugates to yield aglycone and free glucuronate. *E. coli* is among the few bacteria that can synthesize glucuronidase. However, the growth kinetic of *E. coli* has been recognized as 1.9804 h^{-1} at 40°C. The gene of thermophilic *β*-glucuronidase enzyme from a thermophilic microorganism can be cloned into *E. coli*. This is important to avoid common problems that associated with high temperature anaerobic fermentation process to increase the yield and productivity. On the other hand, the synthesized recombinant protein is intracellular and thus the extraction process is required [1]. Putative *β*-glucuronidase from *Thermotoga maritima* was cloned and expressed in *E. coli* has relatively wide-ranging pH-dependence with activity from pH 4.5 to 7.5 and a maximum at pH 6.5 [2].

MPS VII or sly syndrome caused by *β*-glucuronidase deficiency is extremely rare and only few cases have been reported worldwide. A small sample of cases is available from which to extrapolate mortality figures for MPS VII. Lysosomal storage diseases affect one baby in 7,000 live births. In addition, the sly syndrome collectively causes disabilities in about one in 5,000 births [3]. Fetal deaths have been noted several times. In mild cases, survival to age 19-20 years has been reported. Upper respiratory tract infections, neurodegenerative complications, and gastrointestinal tract conditions may contribute to reduced survival rates. Those diseases account for a significant share of childhood mental retardation and severe, often fatal, disabilities [4]. Because of low percentage number of survival and brain involvement, the production of *β*-glucuronidase become more significant. This work aims to determine the optimum production of *β*-glucuronidase.

Due to the medicinal effects, pharmaceutical products bear a high commercial value. For instance, *β*-glucuronidase enzyme can be sold for up to USD 2900/kg enzyme in powder form [5]. However, a common difficulties associated with this industrial sector is the production of this pharmaceutical is mainly carried out through various stages which often lead to high losses and low product yield. Hence more effort is needed to enhance this industry into a viable and profitable industrial sector. For instance, processing (extraction) technology, process synthesis and optimization, and product formulation are crucial for this transformation.

This work presents the use of a batch process simulator in modeling and optimizing a locally developed process for *β*-glucuronidase enzyme production. The use of computer aided process design and simulation tools is still relatively new to the field of bioprocessing [6]. Modeling and simulation works for biochemical production were only found in the last decade [7-10].

In this work, SuperPro Designer v5.5, a commercial process simulation tool is used to develop an economically viable scheme for the production of *β*-glucuronidase enzyme. The base case simulation model is based on pilot scale experiments which was up-scaled from laboratory scale. All process condition has been optimized (not reported) to achieve industrial scale production, increased production is needed for the base case process. Hence, various process and scheduling bottlenecks needs to be overcome. Seven alternative production schemes were further developed by incorporating various debottlenecking strategies. Results reveal that the industrial scale production scheme is good economic performance, with a return on investment (ROI) of 31.62% and a payback period of approximately four years.

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Materials and Method

Base case process simulation

Figure 1 shows the simulation flowsheet of the base case model, developed in SuperPro Designer v5.5. The three main processing steps in this enzyme production consist of fermentation, recovery and purification processes. The current process is operated at an annual batch throughput of 8,523 kg of β-glucuronidase enzyme, which is supplied in powder form. The detailed processing steps are explained as follows.

In the fermentation section, the intial bacteria recombinant E.coli carrying β-glucuronidase from T.maritime is prepared and transferred from a freezer (-800CD) in to asterilized shake flask (SFR-101) containing media, where the cultures undergo the first ferment stage. The fermented products are then sent to four subsequent fermentation steps, carried out in a 5 L seed fermenter (V-101), 50 L and finally 500 L fermenters (V-103 and V-104). Media for V-103 and V-104 are prepared by the media blending tank (V-102) and the heat sterilizer (ST-101).

Cultures from V-104 are harvested by centrifugation (CF-101) before they are transferred to the blending tank V-105 to be mixed with phosphate buffered saline (PBS). Cultures from the blending tank are then homogenized (in HG-101) and undergo a series of filtration steps, i.e. micro filtration (MF-101), ultra filtration (UF-101) and diafiltration (DF-101) to produce the concentrated β-glucuronidase enzyme.

The concentrated enzyme from the diafiltration is transferred to ion exchange chromatography (INX-101) and dead end filtration (DE-101) for final purification. Then the product is then sent to a freeze dryer (FDR-101) where the enzyme is produced in powder form.

As the manufacturing process is carried out in batch operation, efforts have been made to document the scheduling details of each processing steps. This includes the setup time (SUT), process time (PT), and start time (ST) of each individual operation in each unit procedure. SUT is the preparation time needed before an operation takes place. Often, this involves the loading of raw material (e.g., from loading area), equipment preparation or setup that often occur in batch processing. PT on the other hand, represents the actual processing duration needed for each operation. Finally, ST documents the beginning of an operation [11]. The details of this scheduling summary are shown in Table 1, with the Operation Gantt Chart shown in Figure 2.

Process throughput analysis is next performed on the base case simulation model. The model reports the batch production of 8.92 kg β-glucuronidase enzyme. Based on the annual operating time of 7920 hours and minimum cycle time of 5 h (the bottleneck process), the annual production for the process model is calculated as 956 batches. This corresponds to an annual production of 8,523 kg of β-glucuronidase enzyme. This production rate is sufficient for the current local demand which is approximately 6,750 kg of annual production.

Preliminary economic analysis is also conducted on the base case model. The raw material price for the case study includes peptone at a purchase price of USD 94.10/kg and fresh water at USD 0.06/kg. In contrast, the final product of β-glucuronidase enzyme powder is sold at USD 2600/kg. Economic analysis reveals that the current production

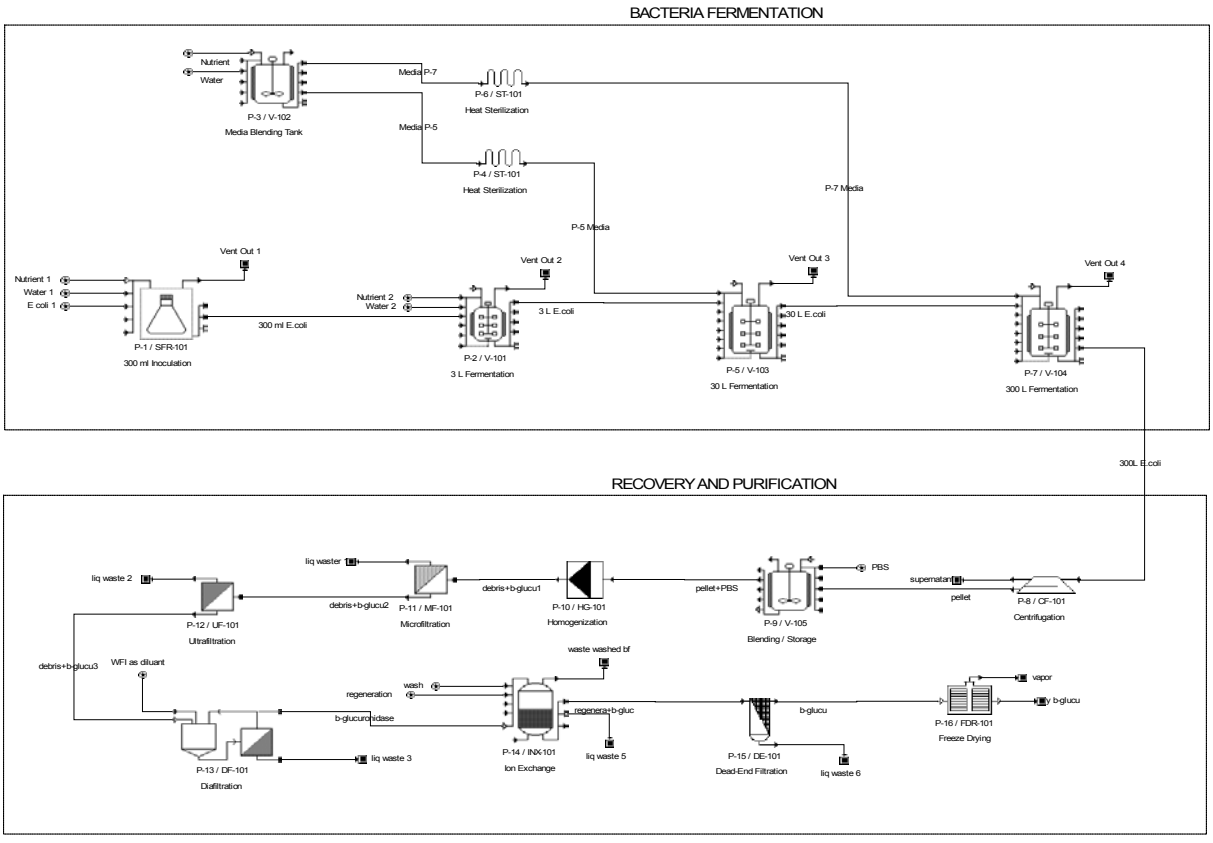

Figure 1: Process flowsheet of the base case model.

scheme has a relatively high capital investment (assuming that all process equipment is newly purchased) and operating cost, as compared to its annual revenue. Its return on investment (ROI) is estimated at 19%. Hence efforts are also needed to improve the economic performance of the production scheme, apart from increasing product throughput.

Results

Throughput analysis

In order to increase process throughput, many factors can be improved or optimized such as batch process control. Another factor should be considered also is process bottleneck. This has been identified can limits the current production. Bottlenecks are process limitations that are related to either equipment or resources such as demand for various utilities, labor, raw material, etc. In batch manufacturing, two types of process bottlenecks may exist, i.e., size bottleneck and time bottleneck. For the former, an index known as capacity utilization may be used to quantify the fraction of equipment capacity usage during an operation. On the other hand, time bottleneck may be quantified using another index known as equipment uptime, which is the measure of how effective a piece of equipment is utilized in time. The product of the two indexes defines the combined utilization of the respective equipment. The processing step with the highest combined utilization is in general the first candidate equipment to become process bottleneck [12,13].

Figure 3 shows the Throughput Analysis Chart that displays the capacity utilization, equipment uptime and combined utilization for each procedure/equipment pair of the base case model of the β-glucuronidase enzyme production. As shown, the freeze drying procedure (P-16/FDR-101) is identified as the process bottleneck due to its highest combined utilization. The capacity utilization of this procedure reaches its maximum 100% while its equipment uptime is relatively high (drying operation duration of 5 h, Table 1). Due to the long operating time, P-16 can also be classified as a process scheduling bottleneck, which limits the annual production of 956 batches. Hence, in order to increase

Procedure	Operation	SUT	PT	ST
P-1/ SFR-101(300 ml Inoculation)	CHARGE-Nutrient 1		5.00 min	Beginning of batch
	CHARGE-Water 1		3.00 min	After Nutrient 1 charge
	AGITATE-1		5.00 min	After Water 1 charge
	CHARGE-*E. coli* 1		3.00 min	After Agitation
	FERMENT-1		5.00 hrs	After *E. coli* 1 charge
	TRANSFER-OUT- *E. coli*		3.00 min	After Fermentation
P-2/ V-101(3 L Fermentation)	CHARGE-Nutrient 2		5.00 min	After 5 hours of batch operation
	CHARGE-Water 2		3.00 min	After Nutrient 2 charge
	AGITATE-1		30.00 min	After Water 2 charge
	TRANSFER-IN- *E. coli*		Master-Slave with P-1 Transfer Out of *E. coli*	Starts with Transfer in from P-1
	FERMENT-1		4.00 hours	After Transfer in of *E. coli* from P-1
	TRANSFER-OUT- *E. coli*		3.00 min	After Fermentation
	CIP-1		15.00 min	After Transfer out to P-3
P-3 /V-102(Media Blending Tank)	CHARGE-Nutrient	0 min	Calculated based on 600 L/h	After 5.31 hours of batch operation
	CHARGE-Water	20 min	Calculated based on 600 L/h	After nutrient charge
	AGITATE-1	-	10.00 min	After water charge
	TRANSFER-OUT-Media to P-5	20 min	Calculated based on 600 L/h	After Agitation
	STORE-1	-	4.66 hours	After Transfer Out of Media to P-5
	TRANSFER-OUT-Media to P-7	20 min	Calculated based on 600 L/h	After Store
	CIP-1	-	15.00 min	After Transfer Out of Media to P-7
P-4/ ST-101(Heat Sterilization)	STERILIZE-1	-	15.00 min	Starts with Transfer Out of Media (to P-5) in P-3
P-5/ V-103(30 L Fermentation)	TRANSFER-IN-Media to P-5	20 min	Calculated based on 600 L/h volumetric flowrate	After Sterilize-1 in P-4
	TRANSFER-IN-3 L *E. coli*	-	Master Slave with P-2 Transfer Out of *E. coli*	Starts with Transfer Out of *E. coli* in P-2 (to P-5)
	FERMENT-1	-	4.00 hrs	After Transfer In of *E. coli*
	TRANSFER-OUT- *E. coli*	20 min	Calculated based on 600 L/h	After Fermentation
	CIP-1	-	45.00min	After Transfer Out of *E. coli*
P-6/ ST-101(Heat Sterilization)	STERILIZE-1	-	15.00 min	Starts with Transfer Out of Media (to P-7) in P-3
P-7/ V-104(300 L Fermentation)	TRANSFER-IN-Media P-7	20 min	Calculated based on 600 L/h	After Sterilize-1 in P-6
	TRANSFER-IN-30 L *E. coli*	-	Master Slave with P-5 Transfer Out of *E. coli*	Starts with Transfer Out of *E. coli* in P-5 (to P-7)
	FERMENT-1	-	4.00 hours	After Transfer In *E. coli*
	TRANSFER-OUT-Broth	-	Master Slave with P-8 Centrifuge	After Fermentation
	CIP-1	-	45.00min	After Transfer Out Broth
P-8/CF-101(Centrifugation)	CENTRIFUGE-8	-	20.00 min	Starts with Transfer Out Broth in P-7 (to P-8)
	CIP-8	-	10.00 min	After Centrifuge
P-9/V-104(Blending / Storage PBS)	TRANSFER-IN-Pellet	-	Master Slave with P-8 Centrifuge	Starts with Transfer Out Pellet in P-8 (to P-9)
	CHARGE-PBS	-	3.00 min	After Transfer in Pellet
	AGITATE-9	-	10.00 min	After charge PBS
	TRANSFER-OUT-PBS+Pellet	-	15.00 min	After agitation
	CIP-9	-	15.00 min	After Transfer Out of PBS+Pellet
P-10/HG-101(Homogenization)	HOMOGENIZE-10	-	30.00 min	Starts with Transfer Out of PBS+Pellet in P-9 (to P-10)

Table 1: Scheduling summary for operations and procedures in the base case model.

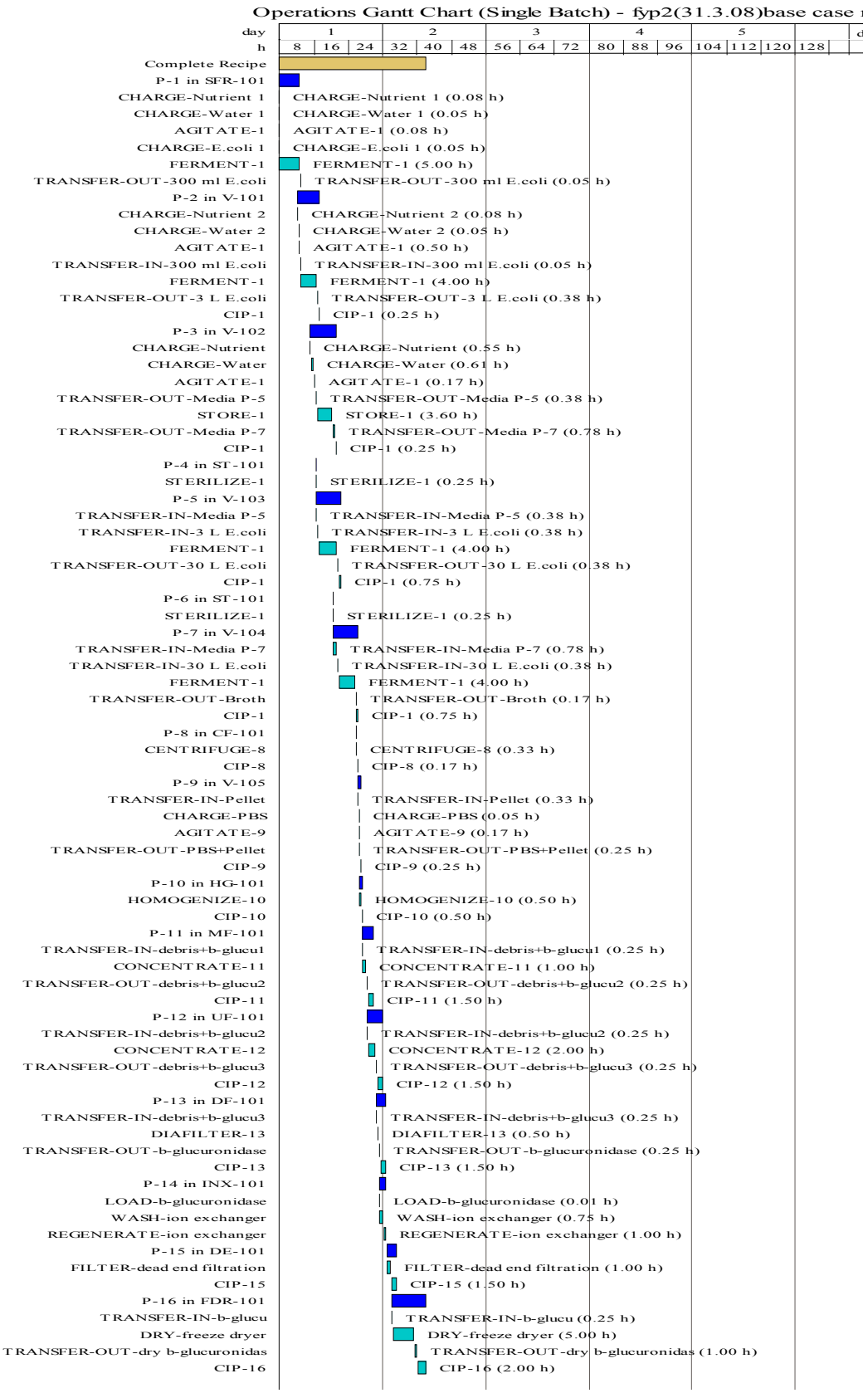

Figure 2: Operations Gantt chart of base case simulation.

the annual production, debottlenecking strategies should focus on the reduction of the drying operation time to enable more batches to be produced annually.

Debottlenecking schemes

The previous section determined that the current production of β-glucuronidase enzyme powder does not reach the desired rate, besides its poor economic performance. Furthermore, efforts to increase production were limited by the process scheduling bottleneck, i.e., freeze drying procedure of P-16/FDR-101. Seven debottlenecking schemes were further developed to evaluate their viability to increase the plant annual production. Economic evaluation was also performed to evaluate all debottlenecking schemes to identify the most economically attractive option.

Alternative debottlenecking schemes

The strategy in Debottlenecking Scheme 1 is to increase the plant throughput by adding an additional freeze dryer (FDR-102). The esti-

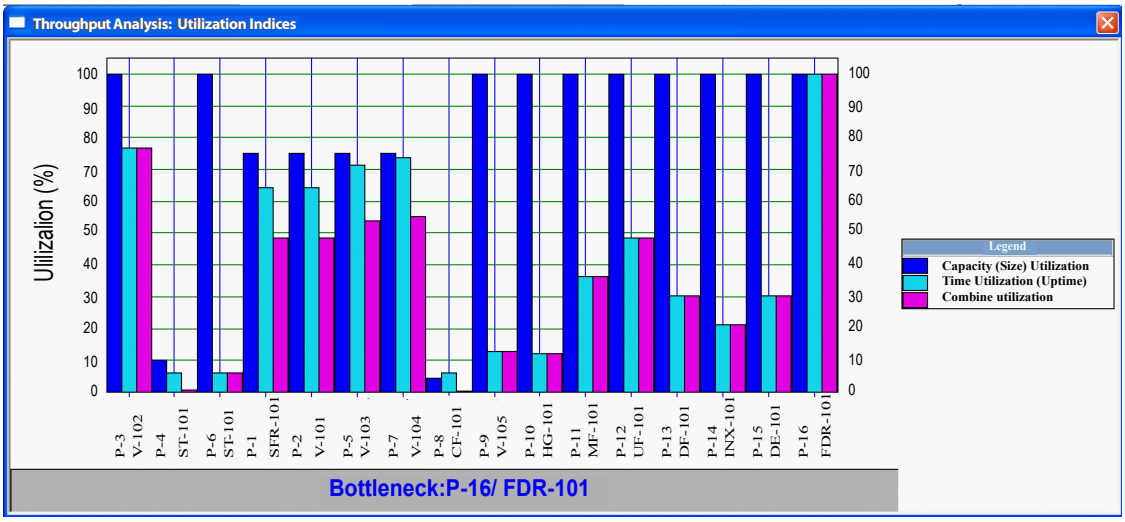

Figure 3: Throughput Analysis Chart for base case simulation.

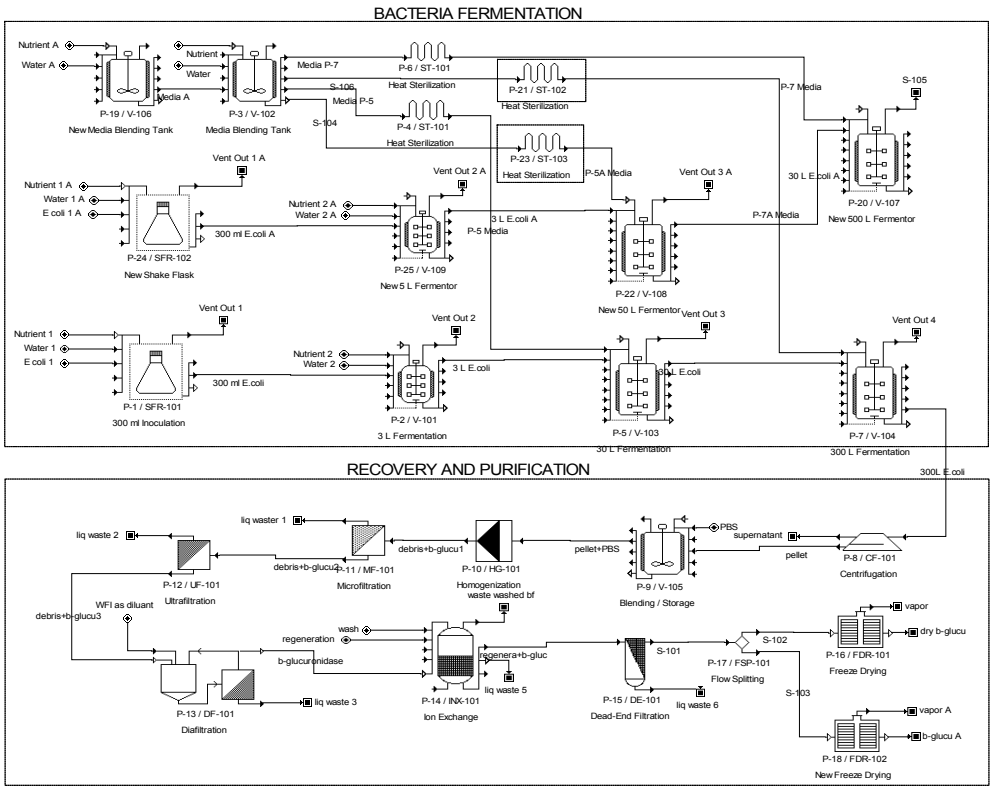

Figure 4: Simulation flowsheet of debottlenecking Scheme 7.

Scenario	Annual batches	Annual Throughput (kg)	Cost of investment (USD)	Annual Operating Cost (USD)	Annual Revenue (USD)	Unit Production Cost (USD/kg)	CBR
Base case	956	8,523.271	14,436,180	19, 650,344	22,160,505	2305.4932	-
Scheme 1	1244	11,090.951	15,292,063	24, 945,062	28,836,473	2249.1364	0.32
Scheme 2	1297	11,563.476	15,667,842	25,958,645	30,065,037	2244.8826	0.36
Scheme 3	1338	11,929.014	16,705,042	26,876,962	31,015,435	2253.0750	0.37
Scheme 4	1484	13,230.685	16,956,818	29,528,347	34,399,780	2231.8079	0.46
Scheme 5	1485	13,239.600	16,958,324	29,546,342	34,422,961	2231.6642	0.46
Scheme 6	1865	16,627.511	17,489,247	36,424,678	43,231,530	2190.6272	0.61
Scheme 7	1912	17,046.543	19,183,343	37,566,787	44,321,011	2203.7775	0.60

Table 2: Throughput and economic analysis results.

mated cost of investment is USD15, 292, 063 and the production cost is reduced to USD 2249/per unit. The simulation result payback time is reduced to 4.22 years. The cost benefit ratio CBR value for this scheme is calculated at 0.32.

Result from Scheme 1 shows that P3/V-102 becomes the new process bottleneck due to its long storage time of media P7. Hence, an additional media blending tank is installed (operates in staggered mode) to remove the bottleneck. Note that Scheme 2 is still using the additional freeze dryer from Scheme 1. Simulation result shows that the annual throughput has increased to 1297 batches, which is 35.7% increment from the base case. The investment cost for Scheme 2 is USD 15,667,842 and the unit production cost is further reduced to USD 2245. The CBR is determined as 0.36, which is higher than that in Scheme 1.

As P3/V-102 is eliminated as process bottleneck in Scheme 2, P-7/V-104 becomes the new bottleneck. Thus in Scheme 3, a new 300 L fermentator is operated in staggered mode with the current fermenter. Freeze Dryer and media blending tank from scheme 1 and 2 remain in Scheme 3. The annual throughput for this scheme is increased to 1338 batches, i.e. 40% increment from the base case. The estimated cost of investment is approximately USD 16,705,042, with the unit production cost slightly increased to USD 2253. The payback time is calculated as 4.29 years, with the CBR of 0.37.

The new process bottleneck that appears from Scheme 3 is P5/V-103, which is the 30 L fermenter with long fermentation time (four hours). In the debottlenecking Scheme 4 which is built on Scheme 3, an extra set of 30 L fermenter is added. The estimated cost of investment is USD 16,956,818, with the unit production cost of USD 2232 and payback time of 3.90 years. Simulation results show that the annual throughput for this scheme is 1484 batches (55.2% increment from the base case), with the CBR value of 0.46.

Scheme 5 for the debottlenecking is next illustrated. As P-1/SFR-101 becomes the new process bottleneck in Scheme 4, an extra 500 mL shake flask is installed to be operated in staggered mode with the current shake flask. Note that Scheme 5 remains all the equipments from previous schemes. The number of batches gradually increased to 1485, which is 55.3% increment from the base case. The estimated cost of investment is USD 16,958,324 with unit production cost of USD 2232 and payback time of 3.90 years. The CBR for this scheme is the same as scheme 4, i.e. 0.46.

Scheme 6 is built based on Scheme 5, where an additional 3L fermenter is installed and operated in staggered mode with the current 3L fermenter. Adding the additional 3 L fermenter overcomes the new process bottleneck that appears in Scheme 5. The annual throughput increases to 1865 batches, which is 95.1% increment as compared to the base case. The estimated cost of investment is USD 17,489,247 with unit production cost of USD 2191 and payback time of 3.16 years. The CBR of this scheme is 0.61 which is higher than that in Scheme 5.

P-6/ST-101 and P-4/ST-101 are now the process bottleneck that needs to be eliminated in Scheme 5. The debottlenecking Scheme 7 is shown in Figure 4 where it is the combination of all previous schemes, with two extra sets of heat sterilizers. The estimated cost of investment is USD 19,183,343, with the unit production cost of USD 2204 and payback time of 3.40 years. Simulation results show that the annual throughput for this scheme is 1912 batches which is 100% increment from the base case, which is the initial target of the production team. Note that the CBR of this scheme is determined as 0.60, which is slightly lower than CBR in Scheme 6.

Table 2 shows the process throughput and economics summary of the seven debottlenecking schemes as compared to the base case simulation model. All debottlenecking schemes demonstrate significant improvement on the annual production. All proposed schemes have seen an increase in capital and operating costs due to addition of new equipments. Among all schemes, Scheme 6 has the highest CBR value which is 0.61 with 95.1% of annual production. On the other hand, the CBR value for Scheme 7 is 0.60 with 100% annual production. Hence, Scheme 7 is identified as the debottlenecking strategy even though Scheme 6 attained highest CBR value; it failed to obtain 100% of production.

Conclusion

β-glucuronidase enzyme production is modeled and optimized in this work based on pilot scale experiment. Seven debottlenecking schemes were proposed and analyzed through simulation. The debottlenecking scheme with the highest throughput that fulfils the customers' need is further analyzed to assess its economic performance. As a result, Scheme 7 is chosen as the best scheme for time debottlenecking to increase the production rate of *β*-glucuronidase enzyme and the one with highest CBR. The modification yields an annual revenue of USD 43M, a gross margin of 15.24%, a return on investment of 29.44% and a payback period of less than four years.

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