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PILOT-PLANT SCALE BIOMASS PRODUCTION BY *LACTOBACILLUS RHAMNOSUS* GG ATCC 53103: A COMPARISON BETWEEN BATCH AND FED-BATCH FERMENTATION

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ABSTRACT

Probiotics such as *Lactobacilli* are important in improving normal intestinal flora and hindering the growth of harmful bacteria in the digestive system. Given the above reasons, the industrial production of probiotics and the use of high-yield strains is of great importance. The present study compares the biomass production by *Lactobacillus rhamnosus* GG ATCC 53103 in batch and fed-batch cultures conditions at a pilot plant scale. An optimized medium containing the following compounds (g/L): glucose 112.50, sugar beet molasses 56.25, casein 18.75, yeast extract 18.75, K_2HPO_4 13.13, Tween 80 1.88, $MgSO_4.7H_2O$ 0.3750, $MnSO_4$. $4H_2O$ 0.0750, $CaCl_2$. $2H_2O$ 0.1875 and Simethicone 1.25 was used for biomass production. During the fermentation process, culture conditions such as pH, temperature, and oxygen concentration were monitored using process analytical technology (PAT). Based on the obtained results, the maximum biomass production in the batch condition in the first 20 hours of culture in the optimized medium was about 68.14 g/L. After three stages of fed-batch culture, the biomass production by *L. rhamnosus* GG ATCC 53103 reached 93.5 g/L at 37°C with agitation and aeration rates of 100 rev/min and 300 $VV^{-1}min^{-1}$, respectively. Therefore, biomass production increased about 2.67-fold more than the basal medium.

Keywords: Biomass production, *Lactobacillus rhamnosus* GG ATCC 53103, Process Analytical Technology (PAT), Batch culture, Fed-batch culture

INTRODUCTION

Probiotics such as *Lactobacillus* strains are present in the healthy human gastrointestinal tract, lowering the pH of the environment by converting sugar to lactic acid (**Ahire *et al.*, 2021**). They have beneficial effects on human health, including lowering blood cholesterol, improving digestive system function, enhancing the immune system, and reducing the risk of colon cancer (**Widyastuti *et al.*, 2021**). The importance of *Lactobacilli* has led to the production of pharmaceutical and related industrial products (**Widyastuti *et al.*, 2021; Marco *et al.*, 2021**). However, the activity and growth of *Lactobacilli* are affected by the culture conditions and medium compositions.

Originally, Sherwood Gorbach and Barry Goldwin isolated *L.rhamnosus* GG ATCC 53103 from fecal samples of a healthy human adult. It is one of the most widely studied probiotic bacteria and, is used in a variety of commercial and industrial probiotic products, due to its resistance to acid and bile, and adhesion capacity to the intestinal epithelial layer. Based on the large number of studies, *L.rhamnosus* GG has been considered as probiotic for human health. Therefore, biomass production and formulation this strain is very useful for large scale probiotic production **(Capurso L., 2019)**. The useful effects of *L. rhamnosus* GG ATCC 53103 have been studied in clinical trials and human studies (**Segers and Lebeer, 2021**).Process analysis technology (PAT), is one of the methods used in the study of bio-productivity, which is an effective and accurate way to identify the best production conditions. PAT is utilized to ensure final product quality by designing, analyzing, and controlling manufacturing through timely measurements of materials and processes by analyzing the effective factors. PAT also helps to produce a uniform and quality product and take an effective step in reducing costs and energy loss, and it is a good option for simplifying the production process.During manufacturing, process parameters are adjusted to produce the desired quality attributes of the process endpoint. The application of PAT can be at-line (sample removal, isolation, and analysis close to process stream), on-line (sample removal from the process stream and returned to process stream), in-line (sample not removed but analyzed in place), and off-line (sample removal and analyzed away from process stream). In PAT, there is necessary for the analytical results to be available in the period necessary for real-time decision-making. The control phase involves designing a control scheme based on the process understanding and consistent process performance and product quality can be achieved (**Rathore *et al.*, 2010; Food and Drug Administration, 2004**).

Several studies have been performed regarding the optimization of *L.rhamnosus* GG 53103 biomass production in different conditions such as carbon and nitrogen sources, growth factors (amino acids and vitamins), and culture conditions including temperature, pH, and aeration in fermentation processes (**Amanda *et al.*, 2017; Kim *et al.*, 2021; Oleksy-Sobczak and Klewicka, 2020; Alvarez *et al.*, 2010; Armand *et al.* 2020**). However, less attention was paid to the comparison of biomass production of *L.rhamnosus* GG ATCC 53103 in batch and fed-batch conditions at pilot-plant scale production. Therefore, the aim of the present study is the optimization and comparison of the biomass production, substrate consumption, and analysis of different conditions by *L. rhamnosus* GG ATCC 53103 in batch and fed-batch culture conditions at the pilot plant scale. Also, process analytical technology was considered in the biomass production *L. rhamnosus* GG ATCC 53103 in batch and fed-batch culture conditions.

MATERIALS AND METHODS

Microorganism, media, and culture conditions

L. rhamnosus GG ATCC53103 lyophilized vial with starter code SP1 and the Lot Number: C161485A was prepared from SACCO S.r.L Biotechnology Company (Cadarago, Italy). A bacterial lyophilized vial was cultured in (De Man, Rogosa, and Sharpe agar (MRS liquid medium at 37°C for 24 hours). For preculture preparation, 10 ml of primary culture of *L. rhamnosus* GG ATCC53103 grown in MRS broth was inoculated in a 1000ml Erlenmeyer flask containing 200 ml of MRS broth. Incubation was performed at 39 °C for 15h on a rotary shaker (110 rev/min), for obtaining 10^8 to 10^9 CFU/mL of the bacterial cells. In the next stage, 400mLof freshly obtained preculture inoculated in new 3200 mL of fresh MRS broth under the above-mentioned conditions. For the preparation of the initial seed culture at the pilot plant scale, 3600 ml of MRS medium containing 10^8 to 10^9 CFU/mLwas inoculated in a 40 L preculture medium containing the following ingredients (g/L); glucose 20, yeast extract 20, peptone from casein hydrolyzed 20, K_2HPO_4, 2.5, and KH_2PO_4 0.6. Fermentation was performed in a 500L Pars Abad fermenter (FR 500, Tehran/Iran) at 37°C with an agitation rate of 100 rev/min. The basal medium for biomass production in the 1300L fermenter (FR 1300, Tehran/Iran) contained the following ingredients (g/L); glucose 90, sugar beet

molasses 45, casein peptone 15, yeast extract 15, K_2HPO_4 10.5, Tween 80 1.5, $MgSO_4$. 7H2O 0.3, $MnSO_4$. $4H_2O$ 0.06, $CaCl_2$. $2H_2O$ 0.15 and Simethicone1.0.

Plackett-Berman method for increasing the biomass production by *L. rhamnosus* GG ATCC53103

Plackett–Burman (PB) method using Design-Expert software 12.0.3.3 (Stat-Ease, Minneapolis, MN, USA) was applied for finding the most significant factors for the biomass production by *L. rhamnosus* GG ATCC53103. The levels of components of the medium in an L_{16} experimental array are shown in Table 1.

Table 1 Medium components levels for optimization of biomass production by *L. rhamnosus* GG ATCC53103

	Factor levels		
Factors (g/L)	Lower	Medium	Higher
Glucose	45	90	180
Sugar beet molasses	22.5	45	90
Casein peptone	7.5	15	30
Yeast extract	7.5	15	30
K_2HPO_4	5.25	10.5	21.0
Tween 80	0.75	1.5	3.0
$MgSO_4$. $7H_2O$	0.15	0.3	0.6
$MnSO_4$. $4H_2O$	0.03	0.06	0.12
$CaCl_2$. $2H_2O$	0.075	0.15	0.3
Simethicone	0.5	1	2

Batch fermentation using the optimized medium

Batch fermentation was performed in a 1300L Pars Abad fermenter (FR 1300, Tehran/Iran). For initial fermentation, 40L of obtained preculture in the 500L fermenter was inoculated in 600L of optimized medium containing the following ingredients (g/L); glucose 112.50, beet molasses 56.25, casein 18.75, yeast extract 18.75, K_2HPO_4 13.13, Tween 80 1.88, $MgSO_4$. $7H_2O$ 0.3750, $MnSO_4$. $4H_2O$ 0.0750, $CaCl_2$. $2H_2O$ 0.1875 and Simethicone1.25. Incubation was performed at 37°C with agitation and aeration rates of 100rev/min and 300 $VV^{-1}min^{-1}$, respectively. The pH of fermentation was kept fixed for 48h at pH 7.0. For determination of glucose, 30ml of samples were taken at 4 hours intervals.

Fed-batch fermentation

The fed-batch culture was started with initial glucose of 112.50 (g/L). The culture conditions including starting pH, temperature, agitation, and aeration rates were the same as batch culture in the 1300L Pars Abad fermenter (FR 1300, Tehran/Iran). The working volume of the fermentation medium was 600L. The pH and dissolved oxygen were monitored during the fermentation process using an optical dissolved oxygen sensor (Visi Ferm, Hamilton-Switzerland) and pH sensors (Arc EasyFerm Plus Hamilton, Switzerland). The pH control was performed by re-alkalizing the culture up to the initial pH of 6.8 with 10N NaOH for every 15 hours of the fermentation process. The feeding substrate was added same time as re-alkalization. The necessary volume of fresh substrate to reestablish the initial total sugars concentration in the fermentation medium were calculated by using mass balance equations for the whole sugars as described by Kim et al. (**Kim *et al.*, 2021**).
In these equations, the volumes of NaOH added to the fermenter in each re-alkalization cycle were considered. Fed-batch fermentation was performed in three stages. In all stages, of fed batch culture, the fermenter was fed with fresh culture medium with initial glucose concentration [S_0] =112.50 (g/L). Feeding and re-alkalization strategies were repeated every 15 hours.

Analytical measurements

In batch and fed-batch culture conditions, the determination of cell growth and glucose consumption was performed according to the method suggested by Oleksy-Sobczak and Klewicka (**Oleksy-Sobczak and Klewicka, 2020**). The growth rate was measured by measuring at OD_{560} nm using a 2100 UV/VIS spectrophotometer (UNICO, USA). Absorbance values were related to the dry weight of biomass estimated from 200ml freeze-dried samples. A polynomial equation was used to measure experimental biomass production.

$$rx = d[X]/dt = \mu [X]$$

where (rx = d[X]/dt) is the experimental biomass production rate, and [X] is the biomass concentration at any given time, *t* is time,
and μ is the specific growth rate. For validation of the results, all experiments were performed in triplicates. For glucose concentration determination, 20 µl of the sample was used in Agilent 1260, USA series of high-performance liquid chromatography (HPLC) equipped with (Agilent G7162A, USA) detector (**Alvarez *et al.*, 2010**).
Acidified distilled water containing 3mM sulfuric acid solution with a flow rate of 1mL/min was used as the mobile phase. Oxygen concentration and pH were determined online using optical dissolved oxygen and pH sensors. All parameters in batch and fed-batch cultures were considered in PAT analysis presented in Table 2.

Table 2 Variables used in biomass production by *L. rhamnosus* GG ATCC 53103 in PAT analysis

Process variable	PAT status	Analytical tool	Target of application
Biomass concentration	At-line	UV/VIS spectrophotometer	Biomass determination
Glucose concentration	At-line	HPLC	Analysis of metabolite for control of product formation
pH	online	optical dissolved oxygen sensor	pH control in fermentation
Oxygen	online	pH sensor	Determination of dissolved O_2 concentration in fermentation broth

RESULTS AND DISCUSSION

Growth parameters include biomass production, glucose consumption, and pH in a batch fermentation process using the basal medium shown in Figure 1. Maximum biomass production was about 35.0 g dry weight/L in 30 hours of the fermentation process at pH 7.
For optimized conditions, an L_{16} array was used in the Plackett-Burman design. The experimental conditions for each trial in comparison to the control are shown in Table 3. The maximum biomass production obtained by *L.rhamnosus* ATCC 53103GG was about 72.3 g dry weight/ L in trial 10.
Analysis of the obtained data and the effects of different factors on the biomass production by *L.rhamnosus* GG ATCC 53103 performed using Design-Expert software, and the results are shown in Figure 2 Pareto chart (A) and Half Normal Plot (B). The results showed that sugar beet molasses, glucose, and casein have the greatest effect on biomass production by *L.rhamnosus* GG. Glucose is a simple and most widely used carbon source for microbial growth. Also, glucose, casein, and beet molasses showed synergistic effects on biomass production. Increasing the concentration of glucose with increasing the concentration of two other factors increases the production of biomass. Amongst the factors, yeast extract showed the minimum effect on biomass production, and a lower level of this medium component can be used.
Other factors such as Tween 80 and Simethicone had positive effects on biomass production. Amongst the mineral sources, magnesium sulfate had the most effects on biomass production. Fermentation was performed according to the suggested levels of medium components provided by Design-Expert software as shown in Table 4.

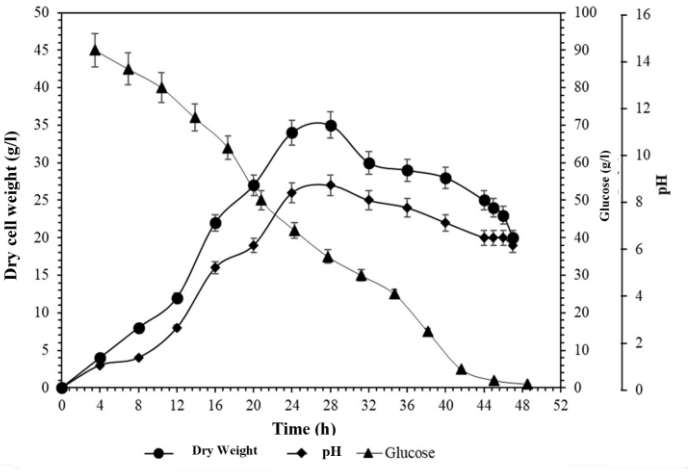

Figure 1 Time course of *L. rhamnosus* GG ATCC 53103 biomass production in basal medium.

Maximum biomass production by *L.rhamnosus* GG ATCC 53103 was 73.4 dry weight /L, in 22 hours of incubation time at pH 7, which was two-fold more than the basal medium as shown in Figure 3. The obtained experimental results were significant at 95% level with the expected results. After optimization, the production medium contains the following compounds (g/L): glucose 112.50, sugar beet molasses 56.25, casein 18.75, yeast extract 18.75, K_2HPO_4 13.13, Tween 80.88, $MgSO_4$. $7H_2O$ 3750.0, $MnSO_4$. $4H_2O$ 0750.0, $CaCl_2$. $2H_2O$ 1875.0 and Simethicone 1.25 was used for biomass production in further studies.

Table 3 Assignment of experimental medium factors and their results of the L_{16} optimization of biomass production by *L. rhamnosus* GG ATCC 53103 according to Plackett–Burman (PB) method. Values are mean ±S. D of triple replicates (p<0.05).

Trials	Glucose (g/L)	Molasses (g/L)	Casein (g/L)	Yeast extract (g/L)	K_2HPO_4 (g/L)	Tween 80(g/L)	$MgSO_4$ (g/L)	$MnSO_4$ (g/L)	$CaCl_2$ (g/L)	Simethicone (g/L)	Biomass production (g/L)
1	180	90	7.5	30	5.25	0.75	0.15	0.12	0.075	2	54.5±0.13
2	45	22.5	7.5	30	5.25	3	0.6	0.12	0.075	2	28.5±0.35
3	45	22.5	7.5	7.5	5.35	0.75	0.15	0.03	0.3	2	27.3±0.41
4	45	90	7.5	7.5	21	3	0.15	0.12	0.075	0.5	42.5±0.18
5	180	22.5	30	30	5.25	0.75	0.6	0.03	0.075	0.5	37.1±0.22
6	45	22.5	30	7.5	21	3	0.6	0.03	0.075	2	25.1±0.71
7	180	90	30	30	21	3	0.6	0.12	0.3	2	58.2±0.47
8	45	90	30	7.5	5.25	0.75	0.6	0.12	0.3	0.5	51.2±0.56
9	45	90	7.5	30	21	0.75	0.6	0.03	0.3	0.5	44.1±0.62
10	180	90	30	7.5	21	0.75	0.15	0.03	0.075	2	72.3±0.48
11	180	90	7.5	7.5	5.25	3	0.6	0.03	0.3	2	52.3±0.39
12	180	22.5	7.5	30	21	3	0.15	0.03	0.3	0.5	38.6±0.39
13	45	22.5	30	30	21	0.75	0.15	0.12	0.3	2	47.6±0.21
14	180	22.5	7.5	7.5	21	0.75	0.6	0.21	0.075	0.5	35.3±0.51
15	180	22.5	30	7.5	5.25	3	0.15	0.12	0.3	0.5	45.6±0.75
16	45	90	30	30	5.25	3	0.15	0.03	0.075	0.5	42.5±0.19
Control	90	45	15	15	10.5	1.5	0.3	0.06	0.15	1	37.0±0.31

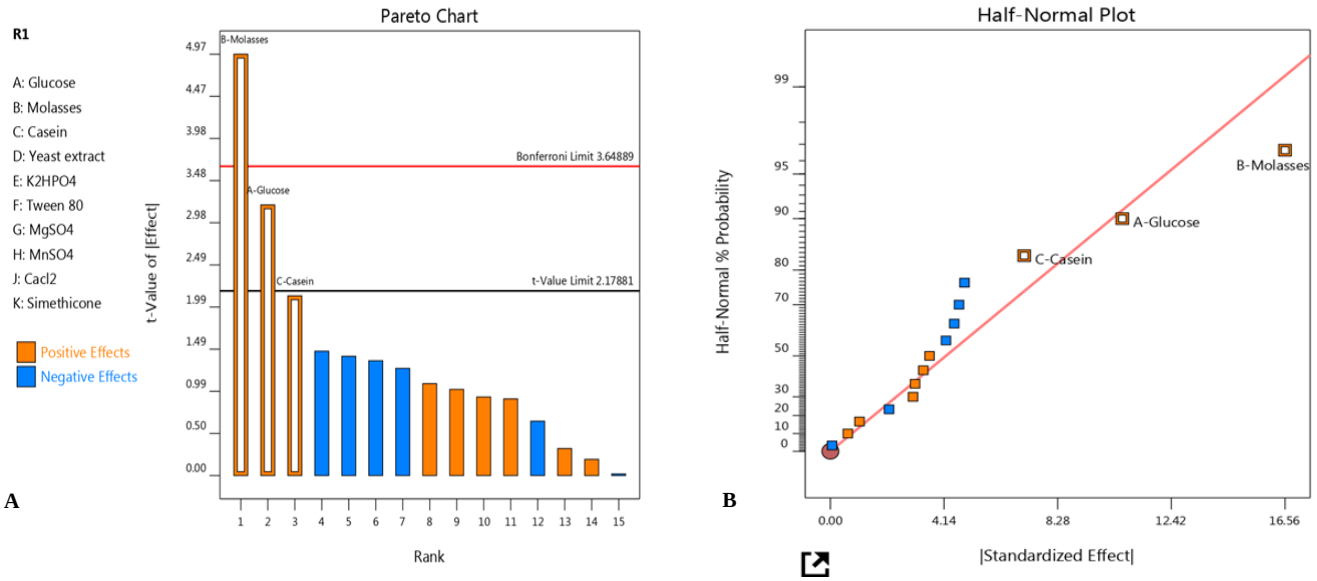

Figure 2 Pareto chart (A) and Half-Normal design or Actual (Standard effects) versus predicted (Half-Normal) plot (B). The F value obtained from this data showed that this model is approved (F equals 7.63), and (p<0.05).

Table 4 Suggested levels of medium components based on Design-Expert software prediction.

Medium component	Glucose (g/L)	Beet sugar Molasses (g/L)	Casein peptone (g/L)	Yeast extract (g/L)	K_2HPO_4 (g/L)	Tween 80(g/L)	$MgSO_4$ (g/L)	$CaCl_2$ (g/L)	Simethicone (g/L)
Optimized level	112.50	56.25	18.75	18.75	13.3	1.88	0.3750	0.1875	1.25

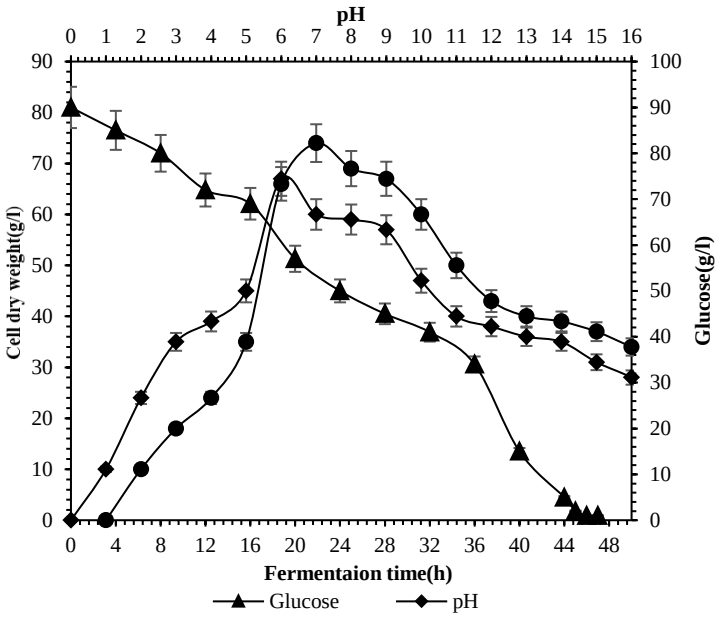

Figure 3 Time course of *L. rhamnosus* GG ATCC 53103 biomass production in optimized medium in batch culture. Values are mean ±S. D of triple determinations.

Biomass production by *L. rhamnosus* GG ATCC 53103 in fed-batch culture conditions

Figure 4. Shows biomass concentration, glucose consumption and lactic acid production in the first 20hours of batch fermentation, before fed-batch culture, a biomass concentration of 68.14 g/L was achieved.

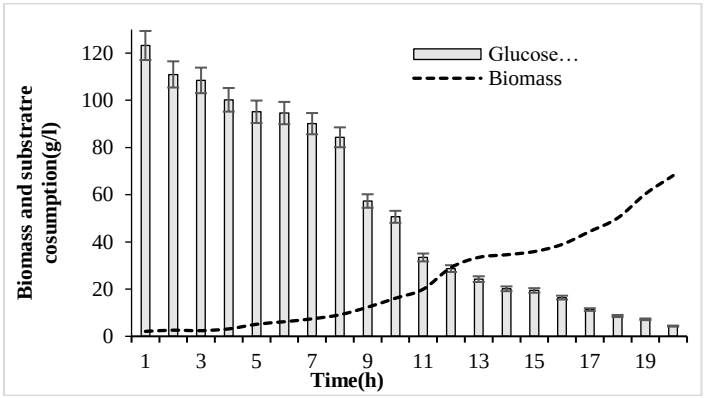

Figure 4 Time course of biomass production, glucose consumption in the first 20 hours of fermentation before fed-batch culture.

In the first cycle of fed-batch, the agitation was stopped and the bioreactor content was allowed to settle for 2 h. Afterwards, 200 liters of fermentation broth was removed from the fermenter and the same volume was replaced with fresh culture medium with a substrate concentration. The fermentation was continued for 15 hours, and the biomass concentration and substrate consumption for this cycle were 76.5(g/L) and 2.46 (g/L), respectively as shown in Fig 5(Fed-batch 1).
In the second fed-batch cycle, without a settling 100 liters of fermentation broth was removed and the same volume of fresh culture medium was added. Again, the fermentation was continued for 15 hours, for the second cycle biomass formation and substrate concentration were 82.54 (g/L) and 2.27(g/L), respectively Fig 5(Fed-batch 2). In the third fed-batch cycle, without a settling 100 liters of fermentation broth was removed and the same volume of fresh culture medium was added. The fermentation was continued for another15 hours, for the third cycle, biomass formation and substrate concentration were 93.5(g/L) and 2.40(g/L), respectively Fig 5(Fed-batch 3). The obtained results showed that the biomass production in the third fed-batch culture increased about 2.67 fold more than the basal medium.

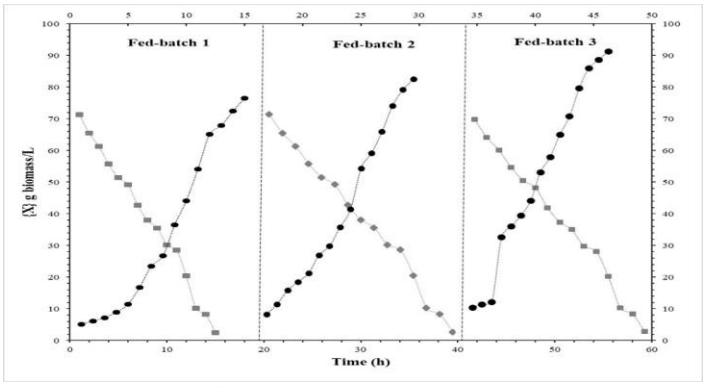

Figure 5 Time course of biomass production and substrate consumption in three fed-batch culture conditions

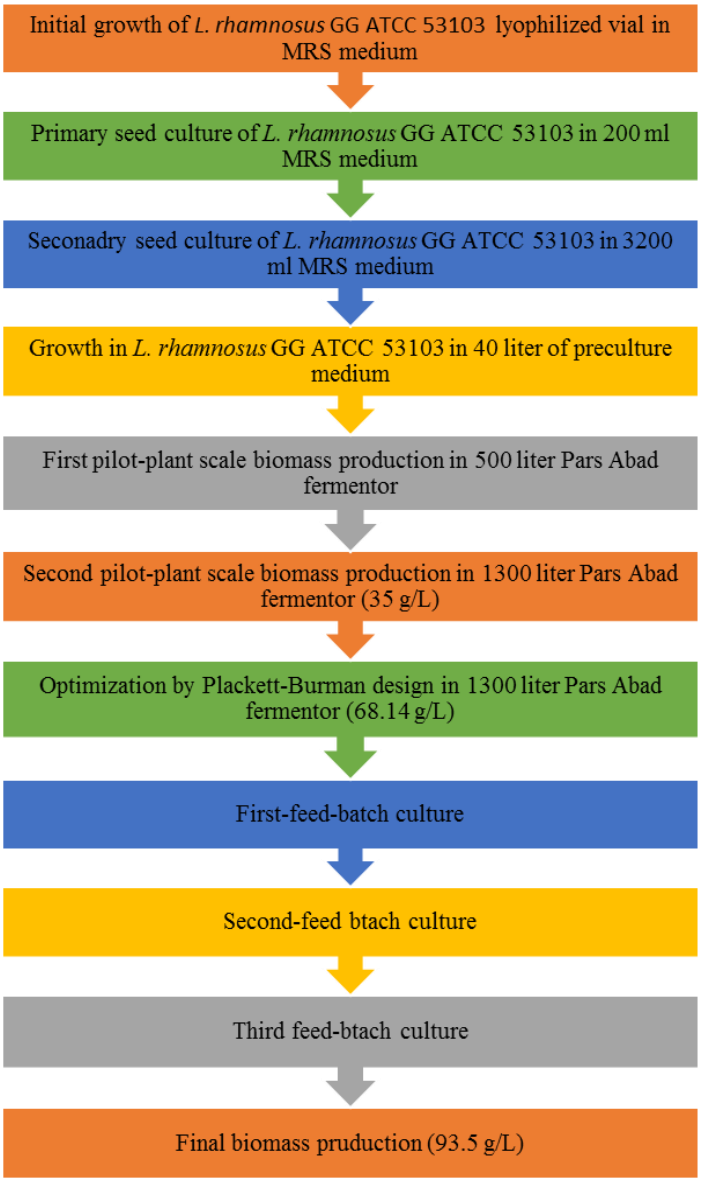

Figure 6 Schematic flowchart of biomass production by *L. rhamnosus* GG ATCC 53103

Using probiotics in different sectors such as the food and beverages industries are very important. Therefore, obtaining the maximum biomass for probiotics is of great importance. Optimization of medium components and culture conditions plays an important role in reaching this aim.
In this study, we used an optimized medium by Plackett-Burman design method from our previous study (**Alvarez *et al.*, 2010**).
In this study, biomass production by *L. rhamnosus* GG ATCC 53103 conditions were compared in 1300-liter pilot-plant scale fermenters (Pars Abad fermenter FR 1300) in batch and fed-batch culture conditions. The optimized medium for biomass production by *L.rhamnosus* GG ATCC 53103 contained the following compounds (g/L); glucose 112.50, beet molasses 56.25, casein 18.75, yeast extract

18.75, K_2HPO_4 13.13, Tween 80 1.88, $MgSO_4$. $7H_2O$ 0.3750, $MnSO_4$. $4H_2O$ 0.0750, $CaCl_2$. $2H_2O$ 0.1875 and Simethicone 1.25.

In batch-culture conditions, we concluded that glucose is the best carbon source for biomass production by *L. rhamnosus* GG ATCC 53103. In addition, glucose showed a synergistic effect with casein from peptone and sugar beet molasses in biomass production and, the need for yeast extract for biomass production is reduced. Therefore, we can use lower concentrations of yeast extract to reduce the price of culture medium in large-scale productions. Other factors such as Tween 80 as a detergent and Simethicone as an anti-acid compound have also been found to be effective in biomass production. Amongst the mineral salts, magnesium sulfate had the best effect on biomass production by this bacterium. Also, the optimization of fermentation conditions in batch and fed-batch conditions were investigated using the PAT method by controlling the pH and aeration conditions. Maximum biomass production obtained under the optimized condition in batch culture was 74.3 g DW/L in the first 22 hours of the fermentation process at pH 7, about 2.12-fold more than the basal medium. Based on the findings in this study, changing the culture from batch to fed-batch condition, improved biomass production was observed by *L.rhamnosus* GG ATCC 53103 to 93.5 g/L about 2.67-fold more than the basal medium. The optimum agitation rate, aeration, and pH are100 rev/min, 300 $VV^{-1}min^{-1}$, and 7, respectively.

Medium optimization for biomass production by *Lactobacillus acidophilus* performed by **Pedram and Ataei (2014)**. Surface response methodology based on the box-Wilson design was used, and based on the obtained results glucose; yeast extract, KH_2PO_4, K_2HPO_4 in the concentration of (g/L) 5-8.75, 36.75-39, 0.1-0.2125, and 0.3925-0.7075 were the best medium components for biomass production, respectively (**Pedram and Ataei, 2014**).

Hwang *et al.* (2008) performed optimization of medium components for biomass production by *L. plantarum* Pi650 using Taguchi and Box-Benken design method. The obtained biomass concentration in this study was about 4.31g DW/L in basal medium. By optimization using Taguchi and Box-Benken design methods, biomass production reached 7.16 and 8.94g DW/L, respectively, about 2-fold higher than the basal medium (**Hwang *et al.*, 2012**). **Chang and Liew (2013)** optimized medium components for biomass production by *L. rhamnosus* GG ATCC 7469. In this study, glucose, yeast extract, and peptone were the best sources for biomass production by *L. rhamnosus* GG ATCC 7469(**Chang and Liew, 2013**).

Bernardez *et al.* (2008) studied biomass production by *Lactobacillus casei* CECT 4043 in batch and fed-batch culture in a culture medium based on whey. Maximum biomass concentrations in the batch and fed-batch culture conditions were 0.33 and 1.7 g/L, respectively **Bernardez *et al.*, 2008**).

Ming *et al.* (2016) studied biomass production by *Lactobacillus salivarius* in fed-batch culture. The results showed that corn steep liquor, KH_2PO_4, and Tween 80 had significant effects on biomass production by *L. salivarius* **(Ming *et al.*, 2016).**

Beitel *et al.* (2020) used low-cost substrates for increasing the lactic acid by *Lactobacillus delbrueckii*. In this study, lactic acid production reached 162 g/L in 48h of fed-batch culture condition. They concluded that lactic acid production on an industrial scale could be considered using low-cost substrates such as molasses and corn-steep liquor (**Beitel *et al.*, 2020**).

Lee *et al.* (2010) studied medium optimization for biomass production by *Lactobacillus acidophilus* A12 isolated from chicken feces using a one-factor-at-a-time and response surface methodology (RSM). The results showed that in one factor-at-a-time experiment, lactose, yeast extract, and $CaCl_2$ were the best sources of carbon, nitrogen, and inorganic salt, respectively. After optimization, the optimum medium components for biomass production were found to be 17.7 g/L lactose, 18.6 g/L yeast extract, and 0.9 g/L $CaCl_2$. Under these conditions, a maximum cell density of 9.33 Log CFU/mL was produced (**Lee *et al.*, 2010**) .

The growth kinetics of probiotic *Lactobacillus* strains in the alternative, cost-efficient semi-solid fermentation medium was studied by **Śliżewska and Chlebicz-Wójcik (2020)**. They showed that the highest bacterial counts were obtained in cultures conducted in the SSF medium with flours to water ratio of 1:1.5 with a pH of 6.0 at 37° C. Subsequently, the growth kinetics were analyzed in both MRS and the SSF media. The newly designed media contributed to the increased duration of the *Lactobacillus* strain's lag phase, which varied from 1.98 to 5.64; nevertheless, the maximum growth rate of the strains was two times higher in the SSF rather than in MRS. The developed medium has the potential to become a new cost-efficient fermentation medium for *Lactobacillus* spp.(**Śliżewska and Chlebicz-Wójcik, 2020**).

Freitas *et al.* (2020) studied biomass production of L. rhamnosus BRM 029693 in feed-batch fermentation. The highest biomass was observed in the simplified MRS medium of about 0.9 g/L with a pH of 4.7 (**Freitas *et al.*, 2020**).

CONCLUSION

As we know, *L. rhamnosus* GG is one of the most widely probiotics, used in the food industry. So, improving the biomass production of this probiotic is considered as grate importance. The results of this study showed that, by optimization of medium ingredients and culture conditions, the biomass production by *L.rhamnosus* GG ATCC 53103 improved in batch and fed-batch fermentations at the pilot-plant scale. By optimizing medium components and culture conditions at the pilot-plant scale, we can obtain biomass production of about 95g/L in fed-batch culture conditions about 2.67-fold more than the basal medium. In comparison to other studies, and as far as we know, this amount of biomass production by *L.rhamnosus* GG ATCC 53103 has not been reported. Schematic flowchart of biomass production by *L. rhamnosus* GG ATCC 53103 shown in Fig 6. Currently, this condition is used for the production of *L.rhamnosus* GG ATCC 53103 biomass in Zist Takhmir pharmaceutical Co. (Tehran, Iran), and can be considered for large industrial scales of probiotic production.

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