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Modeling of microbial biomass production by *Lactobacillus fermentum* 4-17 isolated from Tarkhineh in dairy sludge optimized by response surface methodology (RSM)

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ABSTRACT

Dairy sludge is one of the main and inexpensive wastes of the dairy industry, which contains the nutrients necessary for the growth of lactic acid bacteria (LAB) to produce microbial metabolites, and for its efficient use, optimization of fermentation conditions is critical. In order to optimize the production of dry biomass, three independent variables of dairy sludge concentration (5, 12.5 and 20%), pH (6, 7 and 8) and the inoculation rate of *Lactobacillus fermentum* strain 4-17 (1, 3 and 5%) were used. Before fermentation, the cell growth and morphology of this bacterium were examined in order to determine the function of the growth rate and confirm the presence of bacteria in this medium. The independent variables were optimized using response surface method (RSM) with central composite design (CCD) in order to maximize dry biomass production by this bacterium. The optimization results showed that the maximum amount of dry biomass production related to the optimal treatment included 20% dairy sludge concentration, 1% inoculation rate and pH 8. Also, according to the results, the dairy sludge substrate is suitable for the fermentation of this bacterium. As a result, the effect of dairy sludge bed concentration and pH on dry biomass production by this bacteria was very significant.

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1- Introduction

dairy waste[1] As strong wastewater with high concentrations of biologically required oxygen[2] (BE) and chemical oxygen requirements[3](COD) It is classified [1]. Considering that these effluents contain most of the solid materials in milk, if they are not controlled, they will cause the loss of a valuable product from the dairy industry and in addition cause environmental problems [2]. On the other hand, due to the presence of high concentrations of organic substances, these wastewaters cause acute problems in the urban sewage system [3, 4]. Dairy sludge produced from milk products (milk, butter, yogurt and cheese) is used to a large extent under the influence of the type of system and operation methods, but its main components include carbohydrates, proteins and fats, which are mainly derived from milk. . The main compound in dairy sludge is lactose sugar, which accounts for 14-16% of this effluent. Nitrogen is one of the other compounds found in this wastewater, which originates from the proteins in milk and in two organic forms (protein, urea and nucleic acids) and ions such asNH^4، NO^2 AndNO^3 may and includes 6-8% of this wastewater. Fat and non-dairy substances are other compounds found in dairy sludge, which include 4.7% and 1.5-3% of dairy sludge, respectively [5]. Dairy sludge generally has a volume between 1-0.05% of the volume of milk and 14-16% of it is dry matter, and on the other hand, this effluent has a smaller share of metals and harmful compounds than molasses and sewage sludge. give [6].

From a historical point of view, fermented products are produced to increase shelf life. The countries of the Middle East, especially Iran, have a long history in the production of fermented products. Tarkhina[4] It is one of the fermented foods that has a dairy and grain base and is a rich source of vitamins such as thiamin, riboflavin andB_{12} and is mostly produced in the western regions of Iran. This fermented product is usually produced from a suspension of wheat, buttermilk and turnip, and due to its low moisture content (3-9%) andpH Low (4.0-4.5) prevents the growth of pathogenic and spoilage microorganisms and thus increases the shelf life of the product [7]. In this fermented food, there is the ability to improve health due to the presence and activity of lactic acid bacteria, so that in the old years, this food was used to cure diseases including colds [8].

Lactic acid bacteria due to the production of useful secondary metabolites such as bioactive peptides[5]Bacteriocins[6] and exopolysaccharides[7] They are useful in various industries such as industrial, food and medical microbiology, and on the other hand, they are considered biological preservatives due to increasing the shelf life of food and improving the immune system [9, 10].*Lactobacilli*[8] A very important member of the familyLAB[9] They are rod-shaped, gram-positive, facultative anaerobic, non-motile, non-sporing, catalase-negative and acid-resistant. Growth temperature*Lactobacilli* It is in the range of

[1] - Dairy sludge
[2] - Bological oxygen demand
[3] - Chemical oxygen demand
[4] - *Tarkhineh*
[5] - Bioactive peptide
[6] - Bacteriocin
[7] - Exopolysaccharide
[8] - *Lactobacillus*
[9] - Lactic acid bacteria

2 to 53 degrees Celsius and the optimal temperature for their growth is 30 to 40 degrees Celsius [11, 12]. This genus of lactic acid bacteria is classified into homofermentative and heterofermentative based on fermentation characteristics and bacteria*Lactobacillus fermentum*17-4[10] In the heterofermentative group, it is mandatory and the ability to produceCO_2 from glucose [13, 14]. Probiotics are a group of microorganisms that have the ability to create beneficial effects in the host's body with their high flexibility and ability to modify healthy intestinal microflora. Most of the lactic acid bacteria are placed in this group and the genera*Lactobacillus* And*Bifidobacterium*[11] From the family of lactic acid bacteria, they have the most probiotic activity [15, 16]. Microorganisms that have probiotic properties should have high resistance to bile salts and acid[17]. bacteria *Lactobacillus fermentum* 4-17 is the study bacterium of this research and it was extracted from Tarkhineh fermented food and its probiotic properties have already been proven[18]. This bacteria has the ability to survive inpH It has low bile salt tolerance. Water repellency percentage[12] and the amount of adhesion[13] it to cancer cellsCaco-243 and 8.5% respectively. This bacterium has a high resistance to the digestive environment and has high antibacterial, adhesion and disinfection properties [19, 20].

One of the influential factors in biomass production[14] by bacteria*Lactobacillus fermentum*17-4 Various conditions and factors such as milk sludge percentage,pH and the bacterial inoculation rate. When many factors are involved in the production of a product, the response surface method[15] (RSM) It is considered an efficient tool to optimize the conditions. This scheme can provide the most suitable information with minimum time and fewer tests. This method has been used in many biological products based on biotechnology, such as biomass, enzymes, antioxidant compounds, etc.[21]. The aim of this study is to optimize the culture medium of dairy sludge as a cheap substrate containing nutrients for growth*Lactobacillus fermentum* 4-17 is obtained from clearing and production of dry biomass by the response surface method. In this study, in addition to the optimization of dry biomass production, the cell growth of this bacterium in the dairy sludge bed and the morphology of this bacterium are investigated.

2- Materials and method

1-2- Activation of bacteria*Lactobacillus fermentum* 17-4

bacteria*Lactobacillus fermentum* 4-17 was prepared from the microbial collection of the Department of Food Industry, Faculty of Agriculture, Ferdowsi University of Mashhad, and on the environmentMRS Agar was cultured at 37°C for 24 hours. Cultivation was done on solid medium in order to determine the contamination, and after ensuring the absence of contamination, the single colony obtained from the mediumMRS Agar to mediumMRS The broth was transferred and incubated in the mentioned conditions [22].

[10] - *Lactobacillus yeast*4-17
[11] - *Bifidobacterium*
[12] - Hydrophobicity
[13] - Adhesion level
[14] - Biomass
[15]- Response Surface Method

2-2-Preparation of dairy sludge as the main substrate for bacterial growth

Dairy sludge is created in the milk fat separation stage by the separator, and the separator device works based on centrifugal force. In this study, dairy sludge was prepared from Razavi Pegah Khorasan factory and in order to reduce the microbial load and precipitation of insoluble proteins in it,pH Its primary in the range of 6 byHCL It was set and heated at 90°C for 5 minutes. In the next step, in order to change the morphology, physicochemical properties and homogenize the compounds in it with spray dryer.[16] Donazle (Sorush Sanat - Neishabur) was powdered with an inlet temperature of 180 degrees Celsius and a feed inlet flow of 44.5 ml/min [23]. Preparation of powder from these wastes, in addition to creating stability, increases the surface area and volume of particles and provides more access for microorganisms to the nutrients in the substrate. To prepare the concentrations used in this research, the determined amount of dairy sludge powder is weighed and dissolved by distilled water [24].

2-3- Measurement of cell growth

strain*Lactobacillus fermentum* 4-17 was investigated for growth ability in dairy sludge culture medium which has the lowest percentage of dairy sludge (5%). In the initial stage, 100 microliters of the suspension containing activated bacteria were inoculated into 5 ml of autoclaved dairy sludge powder at 121°C and 15 minutes, and once every hour for 8 consecutive time intervals in the amount of 100 microliters in a plate containing the medium.MRS Agar was cultured and incubated for 24 hours at 32°C, and in the final stage, the number of colonies in each plate was counted at each time interval [25].

4-2- Examining morphological characteristics of bacteria*Lactobacillus fermentum*17-4

In this test, to check the appearance characteristics of each colony after gram staining by light microscope[17] Was performed. In the next step, samples were taken from similar colonies of Lactobacillus with gram-positive, spore-free and rod-shaped characteristics, and catalase test was also performed to confirm it. In order to achieve higher growth and purity, several times this strain in the environmentMRS It was passed [26].

2-5- Preparation of half McFarland concentration of bacteria*Lactobacillus fermentum* 17-4

To determine the bacterial inoculation rate*Lactobacillus fermentum* 4-17 In the dairy sludge environment, in order to optimize the production of dry biomass, the concentration of bacteria should be adjusted by the McFarland half standard. Nim McFarland standard is prepared by adding 99.5 ml of 1% sulfuric acid and 0.5 ml of 1.175% barium chloride. Correct standard turbidity density using absorbance measurements in a spectrophotometer[18] Characterized. Half McFarland's solution of equivalent turbidity with a bacterial suspension containing CFU/ml10^8creates 1.5 × 530 wavelength [27].

[16] - Spray dryer

[17] - Optical microscope

[18] - Spectrophotometer

6-2- Optimizing the growth of bacteria*Lactobacillus fermentum* 17-4 to the response surface method

The response surface method is one of the most common methods to investigate the combined effect of several variables in order to find the optimal conditions for a multivariable condition. In this study, to investigate the effect of three independent variables[19] Milk sludge percentage, inoculation rate[20] bacteria andpH on the dependent variable[21] (Dry biomass production rate) from the central composite plan[22] The response level method was used and in this design, each of the independent variables were used in three levels (-1, 0 and +1), which allowed second-order polynomial fitting.[23] (Table 1) [28, 29]. A central cube design with 20 runs[24] It was done to evaluate the variables and presented in Table 2. The significance of the linear, quadratic effect and the interaction of the three independent variables investigated on the dependent variable (response) using analysis of variance(ANOVA) evaluated. To determine the correlation of the response obtained from the experimental tests to the independent variables, a quadratic model equation was fitted.

Table1 The levels of the independent variables in the CCD

Variables	Unit	Symbol	High level (+1)	Intermediatelevel (0)	Low level (-1)
Dairy wastwater	**%**	**A**	**20**	**12.5**	**5**
Inoculation	**%**	**B**	**5**	**3**	**1**
pH		**C**	**8**	**7**	**6**

[19] - Independent variable
[20] - Inoculation rate
[21] - Dependent variable
[22] - Central composite design
[23] - Fit second‐ order poly‐ nomials
[24] - Run

Table 2 Experimental design to optimize the fermentation environment of *Lactobacillus yeast* 4-17 for biomass production

Run	Dariy wastewater (%)	Inoculation (%)	pH	Biomass (g/L)
1	20	5	8	20.73
2	12.5	3	7	15.04
3	5	3	7	11.95
4	12.5	3	7	14.68
5	12.5	3	7	16
6	12.5	3	8	22.71
7	12.5	3	6	15.97
8	20	5	6	19.71
9	12.5	3	7	16.87
10	12.5	3	7	14.08
11	12.5	3	7	14.2
12	20	1	8	26.53
13	20	1	6	18.75
14	20	3	7	18.04
15	5	1	8	20.14
16	12.5	1	7	14.93
17	5	5	8	16.07
18	5	1	6	12.36
19	5	5	6	15.41
20	12.5	5	7	13.38

7-2- Measurement of dry biomass

To determine the amount of dry biomass, first, the fermented medium in Falcons should be centrifuged at a speed of 4000xg for 15 minutes at a temperature of 4°C. Supernatant[25] It is removed from the falcones and the remaining cell sediment at the bottom of the falcon is washed twice with distilled water and dried in an oven at 80°C until a constant weight is reached. The weight of dry biomass was expressed as grams per 100 ml [30].

3. Results and Discussion

3-1- Examining the rate of bacterial growth*Lactobacillus fermentum* 17-4 in the dairy sludge bed

In Figure 1, the standard curve of bacterial growth in the dairy sludge bed is drawn based on the number of cells in terms of time, and due to the high turbidity caused by the growth of this strain, it was not possible to check the growth based on light absorption by the spectrophotometer, and the surface culture method Cell growth was evaluated. The growth rate of this strain in the dairy sludge substrate increased in the initial period (up to 4 hours) because the main components of the dairy sludge, as mentioned, is the sugar lactose, which is one of the best nutritious substrates for the growth of this bacterial strain. However, in the period of 4 hours later, the growth rate of this strain decreased, which was due to the

[25] - Supernatant

reduction of nutrients (carbon and nitrogen sources) for the growth of this strain of bacteria, and it entered the stationary phase [31]. Fallah et al. (2018) from dairy sludge bed for bacterial growth*Lactobacillus brevis* [26]PML1used and the production rate of gamma-aminobutyric acid[27] were investigated in this cultivation environment. The maximum growth rate of this strain was in the initial period, and after that, this strain entered the stationary phase due to the limitation of carbon and nitrogen sources [6]. In another study, Eskandari et al. (2015) of dairy sludge bed for growth*Radiobacter rhizobium*[28] and exopolysaccharide production, the maximum growth rate of this strain was observed in the logarithmic phase (early hours) and due to the lack of carbon, nitrogen and phosphate resources, after the early hours, this strain entered the resting phase [32].

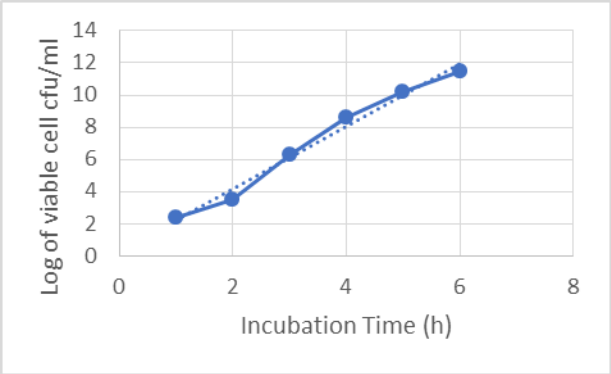

Fig 1 The logarithmic curve of the number of cells in terms of incubation time

2-3- Examining morphological characteristics of bacteria*Lactobacillus fermentum 17*-4

In Figure 2, a view of bacteria*Lactobacillus fermentum* 4-17 is shown separated from the trench. As seen, the colonies of this bacteria were rod-shaped and non-spoza, and according to gram staining, they were placed in the group of gram-positive bacteria, and finally, for final confirmation, they were tested for catalase, and their catalase test was negative. Fallah et al. (2018) also used light microscope images and additional biochemical test of catalase to confirm the presence of bacteria.*Lactobacillus brevis* PML1Use They did[6].

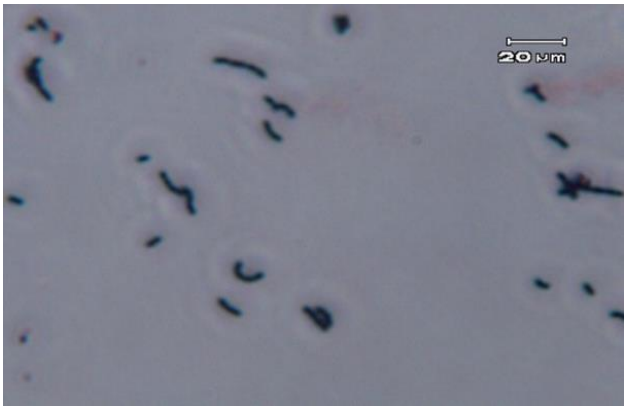

Fig 2 Image taken by light microscope of *Lactobacillus yeast* 4-17

3-3- Optimizing the production of dry biomass by bacteria*Lactobacillus fermentum 17*-4

Response surface method to find optimal operating conditions for dry biomass production from bacteria*Lactobacillus fermentum* Done 4-17. According to the central cube model, 20 runs were performed to optimize three independent variables for dry biomass production, and the design matrix of variables with experimental results is shown in Table 2. Experimental results obtained using the softwareDesign expert Version 13 was installed. The model equation obtained from this software was used to determine the correlation between the dependent variable (response) and the

[26] - *Lactobacillus brevis*PML1
[27] - γ-aminobutyric acid
[28] - *Rhizobium radiobacter*

independent variables. Also, the general equation for predicting the optimal point was explained as follows, in this equationAND،A ،B AndC respectively, equivalent to the amount of dry biomass produced by bacteria*Lactobacillus fermentum* 17-4(g/L), concentration of dairy sludge (%), inoculation rate of this strain (%) andpH are. The effect of different levels of variables on dry biomass production can be obtained by solving equation 1. Also, the current equation shows the influence of each of the factors, their square effects and their mutual effects, and based on that, it is possible to predict how much dry biomass production can be expected as a result of changes in each of the factors.

Eq. 1: Biomass(Y) = 15.07 + 2.78A – 0.7410B + 2.40C – 0.4775AB + 0.0450AC – 1.74BC + 0.0364A² – 0.8036B² + 4.38C²

Data obtained for dry biomass produced by bacteria*Lactobacillus fermentum* 4-17 using this software under one-way analysis of variance (ANOVA) were placed ResultsANOVA For fitting the response surface models are presented in Table 3.

Table 3 Analysis of Variance (ANOVA) of factors affecting dry biomass production

Source	Sum of Square	df	Mean Square	F-value	P-value	
Model	245.24	9	27.25	31.58	< 0.0001	**significant**
A-Dairy sludge	77.45	1	77.45	89.76	< 0.0001	
B-Inoculation	5.49	1	5.49	6.36	0.0303	
C-pH	57.50	1	57.50	66.64	< 0.0001	
AB	1.82	1	1.82	2.11	0.1766	
AC	0.0162	1	0.0162	0.0188	0.8937	
BC	24.08	1	24.08	27.91	0.0004	
A²	0.0036	1	0.0036	0.0042	0.9495	
B²	1.78	1	1.78	2.06	0.1819	
C²	52.79	1	52.79	61.18	< 0.0001	
Residual	8.63	10	0.8628			
Lack of Fit	2.67	5	0.5335	0.4474	0.8010	not significant
Pure Error	5.96	5	1.19			
Cor Total	253.87	19				
R^2	**Adjusted R^2**	**C.V %**	**Adeq Prechsion**			
0.9660	0.9354	5.50	22.5128			

the amount ofp Calculated for the modelQuadratic It was less than 0.0001, which indicates the importance of this model. In this model, the coefficient of explanation(R^2 0.9660) and the corrected coefficient of explanation(R^2adj 0.9363) It confirms that the presented model is very meaningful. In other words, 96.60% of response changes are related to changes in independent variables, and only 3.40% of response changes are not explained by the model. Also, in order for a model to have a good ability to fit information, it is necessary in addition to the coefficient of

explanation(R^2) Above, the lack of fit test[29] That model is also meaningful0.8010) (p-value= not be; Because the non-significance of the lack of fit test of a model indicates the good fit of the used data [33]. In this model, the value of the signal-to-noise ratio[30] is 22.512, which is a very good value for the signal-to-noise ratio. If the valuesp For any variable, if it is greater than 0.05 (at 95% confidence level), it indicates that the variable is not significant in the model, and if it is smaller than this value, it indicates that the variable is significant. According to Table 3, analysis of variance showed that the variables of dairy sludge concentration(A), bacterial inoculation rate(B) And(C) pH It was significant in terms of linear and interaction effectsAB AndAC There is no significant effect, but there is a reciprocal effectBC It was meaningful. Also, the analysis of variance table showed that the second order effects of the variablesA^2AndB^2 It was not significant, while the effect of the second degree variableC^2 It was meaningful. Another factor examined in this parameter tableF-value which shows the validity of the model and the value of the factors used for optimization. This parameter for the model is different from the parameterp-value The higher its value represents the higher value of the model, and on the other hand, the higher the valueF-value If the factors used for optimization are higher, it indicates the greater influence of that factor on the amount of the dependent variable (response) [34].

According to the amountF-value The factors and their effects on the amount of the dependent variable are as follows:

<Concentration of dairy sludgepH Bacteria inoculation rate<

Another parameter considered for the statistical analysis of test data is that the data is normally distributed. To check the normality of the data, you can use the residual normal probability diagram[31] used. If the data is not far from the line drawn in Figure 3, the data distribution is normal and the data has been entered with the least amount of error. Also, in this graph, the closer the data is from blue to red color spectrum, the amount of dry biomass produced by this bacteria increases.

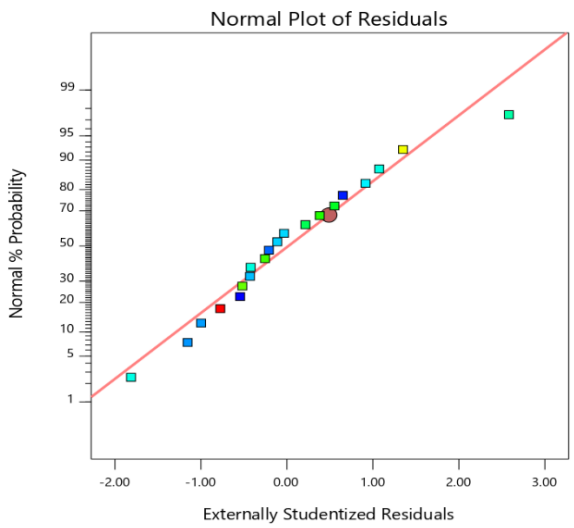

Fig 3 Normal residual probability diagram for dry biomass production

On the other hand, another investigated parameter that confirmed the adequacy of the proposed model was Figure 4, which is based on the number of experiments performed in the external residual value.[32] Was drawn. This graph represents the standard deviation of the actual values from

[29] - Lack of Fit
[30] - Adeq Precision
[31] - Normal plot of residuls
[32] - Externally studentized residuals

the predicted values[33] It is superior to other charts due to checking all the points to estimate the variance. According to this diagram, the proposed model is sufficient for this test, and on the other hand, all the runs in the test are between the red lines.

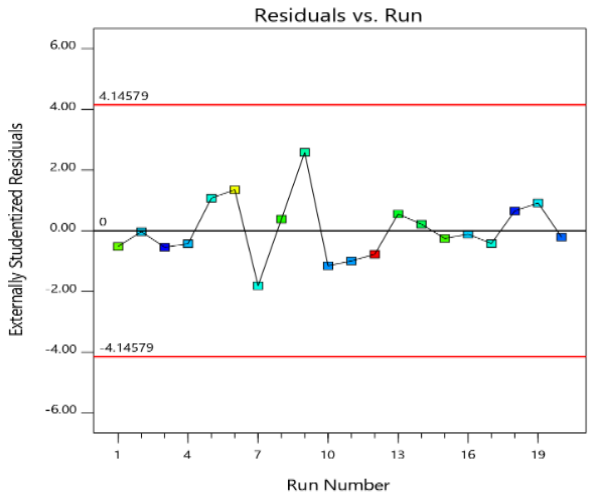

Fig 4 The graph of runs in external residual values

On the other hand, putting the max[34] The production rate of dry biomass as the most optimal answer, the software selected execution number 12 as the best answer, in which the dairy sludge concentration, bacterial inoculation rate andpH It was equal to 20, 1 and 8 respectively.

according to the chart*a* Figure 5. The greatest influence of the dairy sludge concentration factor on the amount of dry biomass produced by bacteria is related to the maximum concentration of dairy sludge because the slope of the line is maximum at the maximum concentration of dairy sludge. Also according to the diagram*b* Figure 5. The greatest influence of the inoculation rate factor on the amount of dry biomass produced by bacteria, corresponding to the minimum[35] The inoculation rate of this bacterium is because the slope of the line is the minimum inoculation rate. On the other hand, according to the diagram*c* Figure 5. The most effective factorpH On the amount of biomass Dry produced by bacteria, related to maxpH is because the slope of the line is at the maximumpH is the maximum

[33] - Predicted values
[34] - Maximum
[35] - Minimum

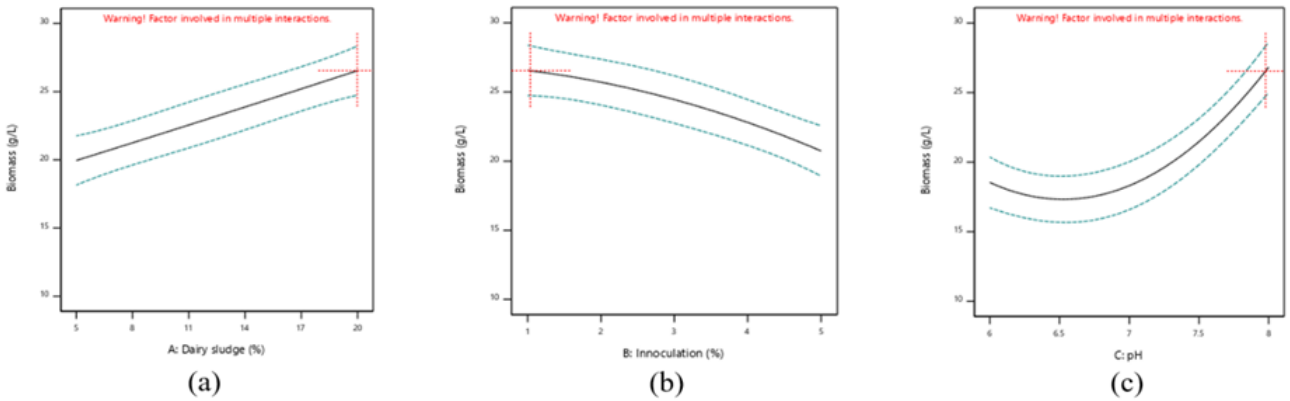

Fig 5 Influence diagram of independent factors on the dependent variable

In forms (6, 7 and 8) the effect of different factors on the production rate of dry biomass by bacteria in two forms of contour diagrams[36] and response level[37] It was checked. In contour plots (a), the response is plotted for two independent factors. While other factors are constant. Also response surface diagrams((b, the mutual effect of independent factors on the production rate of dry biomass is investigated.

In Figure 6, the diagram((a It shows that when the rate of inoculation in the fermentation medium is considered constant, the amount of dry biomass production has gradually increased in proportion to the increase in the concentration of dairy sludge. On the contrary, when the concentration of dairy sludge is considered constant in the graph, the amount of dry biomass production decreases with the increase of the inoculation rate. On the other side of the chart((b Show the interaction effect of dairy sludge concentration and inoculation rate on dry biomass productionGives. Also, if simultaneously the concentration of dairy sludge is increased to 20% and the inoculation rate is decreased to 1%, the dry biomass production rate increases to a greater extent than when both factors are applied alone. Considering that dairy sludge provides energy and the main component for the production of dry biomass. It seems that the higher production of dry biomass with the increase in the percentage of dairy sludge is related to the consumption of dairy sludge in the next part of the culture (stationary phase)[35]. As mentioned, lactose is the main carbohydrate in dairy sludge, which is considered the main source of carbon for biomass production. The reason for the effect of carbon source on biomass production is the high need of this strain for carbon source[36].

Ghazanfari et al.(2023), the effect of independent factors of milk sludge concentration, flour concentration and time in order to optimize the production of L-

[36] - Contour charts

[37] - Response surface graph

glutamate (answer) by a set of lactobacillus bacteria including*Lactobacillus brevis* strainPML1 ، *Lactobacillus plantarum* strain 1058[38]And*Lactobacillus fermentum* strain 17-4 were investigated. The results of this report showed that by increasing the concentration of dairy sludge (20 percent), the amount of production of L-glutamate increases, because dairy sludge as a carbon source and soybean meal as a nitrogen source can be a suitable culture medium for the cheap production of L-glutamate and increase The concentration of dairy sludge increases the carbon source required for the growth of the mentioned bacteria [28]. Moradi et al.(2022), the effect of independent factors of dairy sludge concentration, glucose concentration and time in order to optimize the production of lactic acid (answer) by bacteria*Lactobacillus delbruecki* subspecies*Lactis*strain[39]PTCC1743 They have reviewed. The results of this report showed that increasing the concentration of dairy sludge (18.16 g/liter) increases the amount of lactic acid production, because increasing the concentration of dairy sludge has a significant effect on the cell growth of this strain of bacteria[37]. Fallah et al.(2022), the effect of independent factors of milk sludge concentration, molasses concentration and fermentation time in order to optimize the production of gamma-aminobutyric acid (answer) by a set of Lactobacillus bacteria (*Lactobacillus brevis* PML1، *Lactobacillus fermentum*17-4 and*Lactobacillus plantarum* 1058) were investigated. The results of this report showed that increasing the concentration of dairy sludge (29.27%) increases the production of gamma-aminobutyric acid, because increasing the concentration of dairy sludge has a significant effect on the cell growth of these bacteria due to the source of carbon compounds [38].

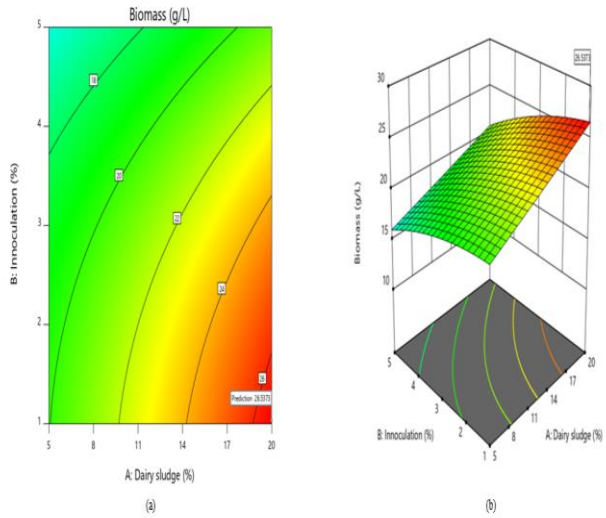

Fig 6 Investigating the effect of dairy sludge concentration and inoculation rate on dry biomass production

In Figure 7, the diagram ((a indicates whenpH In a fixed fermentation environment, the amount of dry biomass production increased gradually with the increase in the concentration of dairy sludge. On the contrary, when the concentration of dairy sludge is considered constant in the graph, the amount of dry biomass production increasespH increase. On the other side of the chart ((b Interaction effect of dairy sludge concentration andpH It shows the amount of dry biomass production. The effect of increasing the concentration of dairy sludge on the amount of dry biomass production is greater than the increasepH It can be concluded that the effect of dairy sludge concentration is more thanpH Is. Also, if the concentration of dairy sludge and pH simultaneously increased to 20% and 8%, the amount of dry biomass production increases to a greater extent than when the concentration of each variable is increased alone. It seems to

[38] - *Lactobacillus plants* 1058

[39] - *Lactobacillus delbrueckii* subsp. *of milk*PTCC 1743

decreasepH It increases the concentration of hydronium outside the cell and thereforepH Foreign decreases. In addition, ifpH If the buffer capacity of the culture medium is exceeded, the biomass will spread back into the cell and due topH It is broken down intracellularly. If the concentration of hydronium ions in the cells*Lactobacillus fermentum* If the buffer capacity of the cytoplasm exceeds, these ions are transported out of the cell through the proton pump, and the cellular energy is stored.(ATP) reduce When energy storage is reduced, the proton pump decrease spH It stops the interior and as a result the growth stops and therefore, the biomass production is also reduced by its nature [39]. Chasvi[40] et al. (2020), the effect of independent factors of carbon to nitrogen ratio,pH The primary cultivation environment in order to optimize productionL-Lactic acid and biomass (answer) by bacteria*Lactobacillus casei*subspecies*Kazai* strainNRRL-441[41] They have reviewed. The results of this report showed that with the increasepH Up to the range (7.5) of the production rate L-Lactic acid and biomass increases becausepH Primary production less than 7.5THE and limits the biomass and it can be justified that whatever pH primary topH The identified containment (3.85) should be closer to biomass production andLLactic acid is stopped faster [40]. Taluri[42] et al. (2017), the effect of independent factors of carbon and nitrogen sources, temperature,pH, stirring speed and incubation time in order to optimize the production of antibacterial compounds (response) by bacteria*Lactobacillus fermentum* MTCC No. 1745They have reviewed. The results of this report showed that with the increasepH By interval (7), the amount of production of antibacterial substances increases because the increasepH Up to 7 intervals, a significant effect on the inhibition of bacteria*Escherichia coli*[43]، *Bacillus subtilis*[44]، *Staphylococcus aureus*[45]، *Pseudomonas aeruginosa*[46] has [41]. Wang[47] et al. (2017), the effect of independent factors of soybean peptone, fermentation temperature andpH primary in order to optimize the production of new exopolysaccharide (Answer) by bacteria*Lactobacillus plantarum* strainKX041[48] They have reviewed. The results of this report showed that with the increasepH Up to the range (6.38) of the production rateEPS increases becausepH Neutral primary for productionEPS It is suitable for alkaline conditions and yieldsdramaticallyEPS reduces[42]. Also, Javad et al. (2013), reported that ifpH Fermentation in biomass production by*Lactobacillus* If it is less than 4.6, the metabolism of microorganisms stops substrate consumption and biomass production [43].

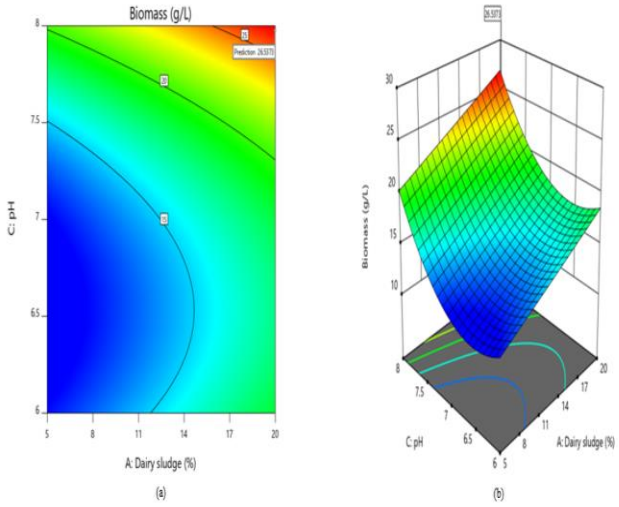

Fig 7 Investigating the effect of dairy sludge concentration and pH on dry biomass production

40 - Chasoy
41 - *Lactobacillus casei* subsp *house* NRRL-441
42 - Ridicule
43 - *E.coli*
44 - *Bacillus subtilis*
45 - Staphylococcus aureus
46 - *Psudomonas aeruginosa*
47 - Wang
48 - *Lactobacillus plants* KX041

In Figure 8, the diagram ((a indicates whenpH In a fixed fermentation environment, the amount of dry biomass production has gradually increased in proportion to the decrease in the inoculation rate. On the contrary, when the inoculation rate is considered constant in the graph, the amount of dry biomass production increasespH, increase. On the other side of the chart ((b Interaction of inoculation rate andpH It shows the amount of dry biomass production. Also, if at the same time the inoculation rate is reduced to 1% andpH increase to 8, the dry biomass production rate increases more than when both factors are applied alone. It seems that the higher the rate of bacterial inoculation in the dairy sludge culture medium, due to the competition and limitation of the substrate required for bacterial growth, the bacteria cannot go through the initial phase of growth and the production of biomass decreases.

Rao[49] et al. (2023), the effect of independent factors on the inoculation rate of a set of lactic acid bacteria including*Lactobacillus paracasei*[50]، *Lactobacillus buchneri*[51] And*Lactobacillus plantarum* and fermentation time in order to optimize the performance of bamboo particles to form glueless particle board[52] They have reviewed. The results of this report showed that fermentation by specific lactic acid bacteria has a higher decomposition rate than bacterial fermentation of lactic acid. When the mixing ratio*Lactobacillus paracasei*، *Lactobacillus buchneri* And*Lactobacillus plantarum* was 1:2:1 and fermentation was carried out for 8 days, improved physical and mechanical properties of particleboard without bamboo glue were obtained [44]. Sunday evenings[53] et al. (2022), the effect of the independent factors of inoculation rate and incubation time in order to optimize the production of bioactive peptide (response) by co-cultivation[54] *Lactobacillus fermentum* strainKGL4[55] and yeast*Saccharomyces cerevisiae* strainWBS2A[56] in a bed of whey protein concentrate[57](WPC-80) They have reviewed. The results of this report showed that increasing the inoculation rate of the co-culture (2.5%) increases the amount of peptide production, because the proteolytic activity of these strains increases with the increase of the co-culture inoculation rate [45]. a blur[58] et al. (2008), the effect of independent factors on bacterial inoculation rate*Lactobacillus delbrueckii*under cheek*Bulgaricus*[59] And*Streptococcus thermophilus*[60] in the form of single culture and co-culture in order to optimize the production of biomass, lactic acid and enzymesb-Galactosidase (answer) was investigated in the medium containing whey. The results of this report showed that the optimal conditions for the production of biomass, lactic acid and enzymesb-Galactosidase, co-culture of two

[49] - Rao
[50] - *Lactobacillus paracasei*
[51] - *Lactobacillus buchneri*
[52] - Binderless particleboard
[53] - Dineshbhai
[54] - co-culture
[55] - *Lactobacillus yeast* KGL4
[56] - *Saccharomyces cerevisiae* WBS2A
[57] - Whey protein concentrate-80
[58] - Taking
[59] - *Lactobacillus delbrueckii* ssp. *bulgaricus*
[60] - *Streptococcus thermophilus*

bacteria*Lactobacillus bulgaricus* And*Streptococcus thermophilus* The ratio is 1.62:1.66. Because lactobacilli can stimulate the mechanism of streptococci through the peptides they produce and the activity of proteolytic enzymes.*Lactobacillus bulgaricus* Than*Streptococcus thermophilus* is more and*Streptococcus thermophilus* can benefit from it and in return*Streptococcus thermophilus* It produces compounds that can grow*Lactobacillus bulgaricus* to influence [46].

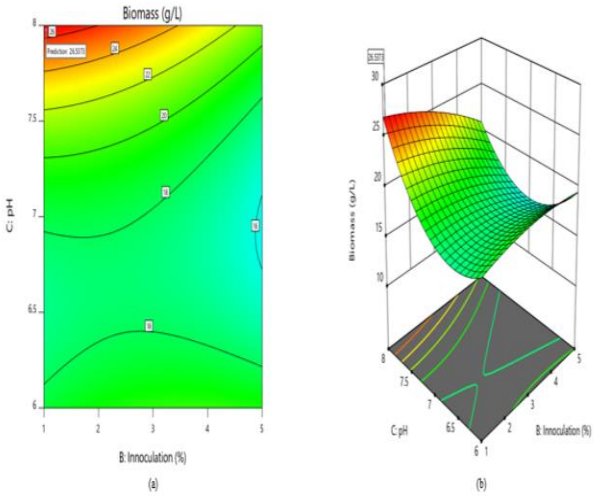

Fig 8 Investigating the effect of inoculation rate and pH on dry biomass production

4 - Conclusion

Bacterial biomass *Lactobacillus fermentum*17-4 strain has useful compounds such as bioactive peptide, bacteriocin, lactic acid, etcL-It is glutamate, for the production of these metabolites using cheap and cost-effective methods, it has received much attention from different researchers. Dairy sludge is a byproduct of dairy factories, which, in addition to being cheap, contains a variety of nutrients such as protein, sugar, fat, vitamins and minerals. By optimizing the fermentation conditions, this bacteria can be used efficiently, for this purpose, the response surface method with central cube design including independent variables of dairy sludge concentration (5, 12.5 and 20 percent),pH (6, 7 and 8) and the inoculation rate of this bacterium (1, 3 and 5%) were used to maximize dry biomass production. The optimization results showed that the maximum amount of dry biomass production related to the optimal treatment includes 20% dairy sludge concentration, 1% inoculation rate andpH It is 8. Effect of dairy sludge bed concentration andpH It was very significant on the production of dry biomass by this bacterium and with increasing the concentration of dairy sludge andpH The amount of dry biomass production increased, and on the other hand, the independent variable of inoculation rate had a negative effect on the amount of dry biomass production. According to the obtained results, it was found that the culture medium of dairy sludge is a suitable environment for the growth of the strain and it can be used on a commercial scale as a suitable substrate.

thanking

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5- Resources

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چکیده

لجن لبنی یکی از ضایعات اصلی و ارزان‌قیمت صنایع لبنی است که حاوی مواد مغذی لازم برای رشد باکتری‌های اسیدلاکتیک (LAB) به‌منظور تولید متابولیت‌های میکروبی است که برای استفاده کارآمد از آن، بهینه‌سازی شرایط تخمیر حیاتی است. به‌منظور بهینه‌سازی تولید زیست‌توده خشک، سه متغیر مستقل غلظت لجن لبنی (۵، ۱۲,۵ و ۲۰ درصد)، pH (۶، ۷ و۸) و نرخ تلقیح باکتری *لاکتوباسیلوس فرمنتوم* سویه ۴-۱۷ (۱، ۳ و ۵ درصد) استفاده شد. قبل از عمل تخمیر، رشد سلولی و مورفولوژی این باکتری به‌منظور تعیین عملکرد میزان رشد و تأیید حضور باکتری در این بستر مورد بررسی قرار گرفت. متغیرهای مستقل با استفاده از روش سطح پاسخ (RSM) با طرح مرکب مرکزی (CCD) به‌منظور به حداکثر رساندن تولید زیست‌توده خشک توسط این باکتری بهینه شدند. نتایج بهینه‌سازی نشان داد که حداکثر میزان تولید زیست‌توده خشک مربوط به تیمار بهینه شامل ۲۰درصد غلظت لجن لبنی، نرخ تلقیح ۱درصد و pH ۸ بود. همچنین باتوجه‌به نتایج، بستر لجن لبنی برای تخمیر این باکتری مناسب است. در نتیجه، اثر غلظت بستر لجن لبنی و pH بر میزان تولید زیست‌توده خشک توسط این باکتری بسیار معنی‌دار بود.