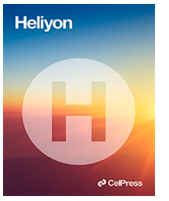
Heliyon 9 (2023) e18744

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



L-Threonine production from whey and fish hydrolysate by *E. coli ATCC® 21277™*

Jhon Fredy Vélez Blandón [a], Claudia Patricia Sánchez Henao [a], José Edgar Zapata Montoya [a,*], Silvia Ochoa [b]

[a] *Nutrition and Food Technology Research Group, Pharmaceutical and Food Sciences Faculty, Universidad de Antioquia, Medellín, 50010, Colombia*
[b] *SIDCOP Research Group, Engineering Faculty, Universidad de Antioquia, Medellín, 050010, Colombia*

ARTICLE INFO

Keywords:
Threonine
Escherichia coli
Agro-industrial wastes
Red tilapia
Box-Behnken design
Culture media design
Essential amino acids

ABSTRACT

In this work production of L-threonine by *Escherichia coli ATCC® 21277™* has been studied using a mixture of alternative low-cost substrates, which are recognized to be a major pollution problem. Whey was used as the primary carbon source, whereas Red Tilapia (*Oreochromis* sp.) viscera hydrolysates constituted the nitrogen source. A Box-Behnken Design was used for optimizing L-threonine and biomass production, using temperature and glucose, whey, and Red Tilapia (*Oreochromis* sp.) viscera hydrolysate contents as factors. Results indicate that biomass production is affected by the concentration of hydrolysate and temperature. On the other hand, L-threonine production is affected by concentration of whey, hydrolysate, and temperature. In this context, it was possible to maximize L-threonine production, but with a detriment on biomass production. The optimal conditions for biomass and L-threonine maximization (after 24 h) were identified and validated experimentally, resulting in biomass and L-threonine production of 0.767 g/L and 0.406 g/L, respectively. This work has shown the technical feasibility of using whey and Red Tilapia (*Oreochromis* sp.) viscera hydrolysates for the production of L-threonine by *E. coli ATCC® 21277™*. Finally, the complications associated to the use of these low-cost complex substrates for the production of L-threonine by *E. coli*, suggest that more in detail studies (i.e. at the metabolic level) are required in order to propose strategies to increase the process productivity, before its scale up. This is a first step in our long-term goal of developing a production process for i) dealing with the pollution problems caused by those wastes, and ii) strengthen the milk and fish industries which are important poles of the Colombian economy.

1. Introduction

L-threonine (THR) is an essential amino acid belonging to the aspartate family [1,2], which helps maintaining a proper protein balance in the body, being fundamental in the synthesis of cellular proteins and enzymes. L-threonine is commonly used in the food as supplement for both, humans and animals [3–5], as well as in the pharmaceutical and cosmetics industries [6,7]. It has also been used as substrate for the production of interesting metabolites, such as propanol [8] and L-2-aminobutyric acid [9]. THR supports the nervous, immune and cardiovascular systems and has a positive effect on the gastrointestinal tract and as promoter of brain activity [10]. The production of THR has associated limitations such as process optimization, production costs, scale-up, strain development,

* Corresponding author.
E-mail address: edgar.zapata@udea.edu.co (J.E. Zapata Montoya).

https://doi.org/10.1016/j.heliyon.2023.e18744
Received 6 March 2023; Received in revised form 11 July 2023; Accepted 26 July 2023
Available online 5 August 2023

List of abbreviations

3,5-dinitrosalicylic acid DNS
Analysis of Variance ANOVA
Biomass X
Biomass production/produced ΔX
Carbon/Nitrogen ratio $R_{C/N}$
Escherichia coli *E. coli*
Fish hydrolysates FH
Glucose GLC
Lactose LAC
L-threonine THR
L-treonine production/produced ΔTHR
Optical density data at 600nm OD_{600}
Red Tilapia (*Oreochromis sp.*) viscera hydrolysate RTVH
Response Surface Methodology RSM
Temperature T

by-product formation, extraction/purification process, among others [11–13]. In 2020, THR market was valued at US$2.05 Bn, and has been projected to grow at 5.47% until 2027 [14]. Microbial THR production has been researched since the 60's, using different microorganisms such as *Escherichia coli (E. coli)* [15–18], *Corynebacterium glutamicum* [19,20], *Brevibacterium flavum* [21], where *E. coli* has been the most used [6,22]. L-threonine is mainly produced by fermentative via, from an economic point of view, on an industrial scale using 50 kL to 500 kL tanks [23,24].

Cow milk processing during cheese production generates a waste product called whey, which is composed mainly by water (93–95%), solids (5–7%), lactose (4.5–5.3%), proteins (0.6–1.1%), fats, salts and minerals [25]. Whey disposal has been pointed out as a threat to the environment and also to human health, because it poses serious pollution problems as it increases the chemical and biological oxygen demand in aquatic habitats [26,27]. Although there are some alternatives for an adequate whey disposal, those are not enough for solving the problem because of the high volumes of whey produced (between 8.5 and 9 L of whey are obtained per 10 L of milk processed). Therefore, new alternatives for dealing with this challenge are still welcome, especially those focused on whey utilization as substrate for metabolites production. For example, the production of recombinant proteins [28], D-tagatose [29], hydrogen [30], ethanol [31], galacto-oligosaccharides [32], THR and L-lysine [33], using whey as substrate has been reported mainly by the action of *E. coli* [28,29,34,35], *Saccharomyces fragilis* [36], *Aspergillus oryzae* and *Kluyveromyces lactis* [32].

Since 2012, the Nutrition and Food Technology Research Group at Antioquia University has been working on looking for alternative uses of the byproducts of the fish processing industry. As this work is part of a project seeking to assess the technical and economic feasibility of the production of essential amino acids, we are incorporating the use of byproducts from the agro-industry, as it is the case of whey and Red Tilapia (*Oreochromis* sp.) viscera hydrolysates (RTVH). In the case of the latter, those byproducts are catalogued as wastes due to its insignificant commercial value [37]. However, the amount obtained of those wastes might be high (between 20 and 80%) depending on the type of fish and processing level [38]. The inadequate disposal of the fish industry wastes constitute another important cause of environmental contamination [39]. In order to reduce such negative impact on the environment, different authors have proposed to treat (by chemical and/or enzymatic action) the proteins present on the fish wastes, in order to obtain fish hydrolysates (FH). Those FH have shown to be safe, to promote therapeutic benefits and to be nutritionally healthy, therefore they have a great potential to be used as ingredients in the pharmaceutical and the dietary supplements industry [40–42]. FH have been reported to be used as substrate in biotechnology processes for lipases production by *Staphylococcus epidermidis* [43] and proteases production by *Pseudomonas aeruginosa* [44]. Other microorganisms such as *E. coli* [45], *Staphylococcus aureus* and *Salmonella enteriditis* [46], have also been explored. Precisely, in this work we use RTVH and whey as substrates for THR production by *E. coli*, in order to evaluate the technical feasibility of using those as raw materials for producing important metabolites (for the food and/or pharmaceutical industry). This would reduce their negative impact on the environment, and at the same time, improve the economy of the agroindustry, by adding some value to those "wastes".

Microbial production of metabolites is affected by operating conditions, such as temperature, pH, agitation and culture media composition [47]. In this context, the composition of the culture media has a direct impact on THR production, as it incorporates basic nutrients such as carbon and nitrogen sources, salts, and in some cases inducers for THR production [13,48] and even microbial growth inducers. Studies reveal that the type of carbon source used has a direct effect on THR production [47]. However, for the case of complex media such as the used in this study, in the open literature, there are no precedents in the production of THR by *E. coli*. In this work, the experimental optimization of THR production by *E. coli ATCC® 21277™* as a function of temperature, and carbon and nitrogen sources was carried out by the response surface methodology (RSM) in a three-step procedure. In the first stage, a preliminary phase was carried out for assessing *E. coli* growth, using whey and RTVH as substrates, since according to the authors' knowledge there are no reports available on the feasibility of this procedure. Second, a Box-Behnken experimental design was performed for optimizing the bioprocess by the RSM. Finally, a validation step was carried out, where the optimal conditions obteined by the RSM were experimentally tested.

2. Materials and methods

2.1. Strain and culture media

Escherichia coli ATCC® 21277™ which has been reported to grow on a medium including L-isoleucine, L-proline, L-methionine and Thiamine and is resistant to concentrations close to 1 mg/mL of α-Amino-β-hydroxyvaleric acid [48], was modified by UV radiation for incrementing L-threonine production. The modified microorganism was directly bought from microbe culture collection of ATCC® in United States.

The microbial growth was carried out using as reference a medium (denoted by M1) composed by 30 g/L of glucose (GLC), 10 g/L of $(NH_4)_2SO_4$, 2 g/L of KH_2PO_4, 1 g/L of $MgSO_4$, 5.4 mg/L of $FeSO_4$, 5.4 mg/L of $MnSO_4$, 0.3 g/L L-proline, 1 mg/L of thiamine, 0.1 g/L of L-isoleucine and 0.1 g/L of L-methionine [48]. The cellular growth was analyzed using different carbon and nitrogen sources in the culture media. Glucose (GLC), lactose (LAC) monohydrate, and Whey were used as carbon source whereas $(NH_4)_2SO_4$ and RTVH were used as nitrogen source. In all the analyzed cases a fixed carbon/nitrogen ratio ($R_{C/N}$) of 6.6 was used as shown in Table 1. These preliminary experiments were carried out in 250 mL flasks containing 100 mL of culture media at 37 °C, pH = 7 and 100 rpm.

2.2. Optimization of the culture media

A Box-Behnken design [49–51] of 29 replicas was developed, having the biomass production ΔX (g/L) and the L-threonine production ΔTHR (g/L) as response variables. The analyzed factors were the concentrations (g/L) of GLC and Whey (LAC), the RTVH (mL/L) and the temperature (T) (°C). Three levels were used for each factor, as it is shown in Table 2.

Levels selection was carried out according to published literature. For example, for *E. coli* growth, T between 5 °C and 45 °C have been reported [52–54]; however, the most frequently reported value has been T of 37 °C [48]. Therefore, 37 °C was taken as the medium level for T, and a variation of 4 °C from this reference value was used for the low and high levels in order to avoid thermal shock [52,54]. To date, no reports were found by other authors studying the effect of pH on THR production by *E. coli*. However, the effect of pH on microbial growth on the *Escherichia coli O157:H7* strain has been reported in Ref. [53], where a correlation between pH and L-threonine production, as well as variables such as the composition of the culture medium and temperature [2,47]. In this work, pH was not considered as a factor due to technical limitations. Therefore, pH was kept at 7, since this is the value reported as optimal for the used strain [48].

On the other hand, while the use of FH was reported for growing *E. coli* [45]; to our knowledge, there are no open literature reports on its use for THR production. Therefore, the lower and higher levels for factor 3 (RTVH content) were selected based on operational. For the case of GLC content, it has been reported that the maximum growth rate for *E. coli* from between 10 g/L and 20 g/L [47,55]. Therefore, the higher level for GLC concentration was taken as 10 g/L, as it was for Whey (LAC).

Culture media were prepared as shown in Table 3, adjusting the $(NH_4)_2SO_4$ content for achieving the desired $R_{C/N} = 6.6$ [48]. Each experiment was carried out during 24 h in 250 mL flasks filled with 50 mL of the culture medium, 100 rpm and pH = 7 regulated with MOPS 100 mM. Samples were collected at 0 and 24 h.

The effect of the different factors on the response variables was evaluated using the Analysis of Variance (ANOVA). The analysis of the results was carried out with the Design Expert® software, as well as the corresponding numerical optimization using the quadratic regression models obtained. The optimum found was experimentally evaluated by triplicate.

2.3. Whey pretreatment

Whey powder was treated following the procedure described by Hausjell with some modifications [28]. The whey was dissolved in distilled water, then sterilized in a Tuttnauer 3870ELV vertical autoclave (Tuttnauer, United States) at 120 °C and 220 kPa. Finally, it was centrifuged at 9000 rpm for 5 min at 4 °C and filtered, maintaining aseptic conditions throughout the process. Lactose content was determined using the DNS method (3,5-dinitrosalicylic acid) [56,57].

Table 1
Culture media composition used in the first stage for assessing *E. coli* growth.

Compound/Media	Concentration (g/L)					
	M1	M2	M3	M4	M5	M6
GLC	30			30		
LAC monohydrate		30			30	
Whey*			30			30
$(NH_4)_2SO_4$	10	10	10	6.94	6.94	6.94
RTVH**				0.13	0.13	0.13

* Lactose concentration.
**The reported values correspond to the atomic nitrogen content in the RTVH.

Table 2
Input information for the Box-Behnken Experimental Design.

	Levels		
Factors	Low	Medium	High
Factor 1: GLC (g/L)	5	7.5	10
Factor 2: Whey (LAC) (g/L)	5	7.5	10
Factor 3: RTVH (mL/L)	200	400	600
Factor 4: T (°C)	33	37	41

Table 3
Box-Behnken Design: Experimental results.

#	GLC (g/L)	Whey (g/L)	RTVH (mL/L)	T (°C)	ΔX* (g/L)	ΔTHR* (g/L)
1	7.5	7.5	600	33	0.939	0.033
2	5	10	400	37	0.934	0.069
3	10	7.5	200	37	1.023	0.111
4	7.5	5	200	37	1.028	0.103
5	7.5	5	400	33	1.021	0.061
6	10	10	400	37	0.997	0.024
7	7.5	10	400	41	0.911	−0.027
8	7.5	5	600	37	0.886	−0.008
9	7.5	7.5	400	37	0.948	0.067
10	10	7.5	600	37	1.046	0.095
11	5	7.5	600	37	1.067	0.059
12	7.5	5	400	41	0.937	0.002
13	7.5	7.5	400	37	0.894	0.127
14	7.5	10	400	33	1.008	0.090
15	7.5	7.5	600	41	0.853	−0.031
16	7.5	7.5	400	37	1.076	0.215
17	10	7.5	400	33	1.056	0.100
18	5	7.5	200	37	1.056	0.219
19	10	5	400	37	1.068	0.162
20	7.5	7.5	400	37	1.068	0.238
21	7.5	7.5	200	33	1.043	0.208
22	7.5	7.5	200	41	1.039	0.136
23	5	5	400	37	1.073	0.026
24	7.5	10	600	37	1.032	0.179
25	10	7.5	400	41	0.863	−0.030
26	5	7.5	400	41	0.916	−0.013
27	5	7.5	400	33	0.996	0.045
28	7.5	7.5	400	37	1.081	0.222
29	**7.5**	**10**	**200**	**37**	**1.042**	**0.310**

*Reported values for ΔX and ΔTHR are calculated as the difference between concentration at t = 24 h and t = 0 h.
Results were analyzed by ANOVA, for determining the significance of the factors on each response variable, using a confidence level of 95%.

2.4. Preparation of the RTVH

Red Tilapia (*Oreochromis* sp*)* viscera were kindly provided by El Gaitero company (Sopetran – Antioquia, Colombia). The viscera were heated to 85 °C for 20 min for inactivating endogenous enzymes. Then, the samples were frozen at −20 °C for 24 h to easily remove the solidified fat by phase separation [58,59]. After that, the viscera were minced using a blender, and packed in PVC bags for further storing at −20 °C. The enzymatic hydrolysis was carried out by Alcalase® action [60] in batches of 500 mL with a protein content of 8 g/L, 700 rpm, 53 °C and pH = 10. When the hydrolysis was completed, the Alcalase® was deactivated by heating at 85 °C during 10 min [58,59]. The hydrolysates were centrifuged at 9,000 rpm and the supernatant was filtered. The protein content was determined by the Kjeldahl method [61]. Amino acids were determined by UHPLC/DAD Thermo Scientific Dionex UltiMate 3000 (Thermo Fisher Scientific, United States) with UV/VIS detector (diode array detector, DAD). Amino acids were derivatized on-line using *o*-phthalaldehyde and 9-fluorenylmethyloxycarbonyl chloride [34,62].

2.5. Analytical techniques

Dry cells weight was determined by gravimetry. Liquid samples were centrifuged at 9000 rpm and dried at 80 °C until constant weight was achieved [63]. Optical density data at 600 nm (OD_{600}) were converted using the factor 0.775 g/L·UA. The OD_{600} of the samples extracted during the fermentations was determined in a Genesys 10 S UV/VIS spectrophotometer (Thermo Scientific, United States). For further analysis, the samples were centrifuged at 14,000 rpm and 4 °C for 10 min [64]. Reducing sugars were quantified in the supernatant using the 3,5-dinitrosalicyclic acid method (DNS) [56,57]. Amino acids were measured by a Thermo Scientific Dionex UltiMate 3000 UHPLC equipment (Thermo Scientific, United States) [34,62].

3. Results and discussion

3.1. Preliminary experiments: E. coli growth from whey and RTVH

E. coli ATCC® 21277™ growth on culture media based on the US3580810 patent [48] was studied, incorporating different substrates (as described in Table 1). Results in Fig. 1 show that when Whey is incorporated to the media (M3 and M6) biomass growth is promoted, reaching 0.656 g/L for the M3 case and 0.810 g/L for the M6 case at the final time (15 h). Furthermore, when RTVH is incorporated to the media as nitrogen source (M4, M5 and M6), independently of the carbon source, a higher biomass (X) production is promoted. The two highest X concentration 0.866 g/L and 0.810 g/L were reached when using media M5 and M6, respectively. These results allow concluding that whey and RTVH are promising substrates for microbial development.

3.2. Culture media optimization

GLC, Whey, RTVH and T were the factors selected for the optimization of biomass (ΔX), the threonine (ΔTHR) produced. For this, a Box-Behnken design was executed, leading to the experimental results shown in Table 3.

3.3. Analysis of factors affecting biomass production (ΔX)

ANOVA analysis shown in Table 4 suggests that the GLC and Whey do not have a significant effect on ΔX. The quadratic regression model obtained is presented in equation (1). Although the adjusted regression coefficient is 0.3439, the lack of fit is not significant and the model is statistically significant (p-value<0.05), then the model can be used in numerical optimization.

ANOVA analysis shown in Table 4 suggests that the GLC and LAC in Whey do not have a significant effect on biomass production. Although the adjusted regression coefficients are low (0.3439, 0.4844 for biomass and L-threonine production, respectively), the lack of fit is a statistical test in which the null hypothesis is that there is no lack of fit. According to Mendenhall et al. [65], and Kutner et al. [66], an adjusted R^2 may not be good, but if the model has no lack of fit, it can be used to make inferences within the range of variation of the factors used. Furthermore, the models are statistically significant (p-value<; 0.05), then the models can be used in numerical optimization. Additionally, the model assumptions regarding the residuals (normality, homoscedasticity and independence) were checked and the three of them were fulfilled for both models (see supplementary material). On the other hand, Adequate Precision statistic measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratios of 7.79 and 8.59 for biomass and L-threonine production, respectively, indicates an adequate signal. These models can be used to navigate the design space [67].

Equation (1) shows the model representing the effect of RTVH and T on ΔX, which can be considered accurate because it fulfills the assumptions of normality, homoscedasticity and independency of residuals [68].

$$\Delta X = -3.08630 - 0.000170 * RTVH + 0.236906 * T - 0.003355\, T^2 \quad (1)$$

The response surface diagram for ΔX as a function of RTVH and T is shown in Fig. 2, where Whey and GLC had values of 7.5 g/L. In there, it is possible to observe that ΔX is favored at temperatures from 35 °C to 37 °C. Biomass growth is suppressed at 33 °C and 41 °C, being this higher at 41 °C. This might be explained by the thermal shock [54]. ΔX decreases when the RTVH content is increased. Therefore, RTVH content must be kept at the minimal value (200 mL/L), because higher values suppress biomass production. This can be explained due to the fact that in general, FH constitute a complex mixture of peptides and free amino acids, being the latter of easy access for the microorganism, which improves ΔX [45], by another hand, antimicrobial activity of FH has been reported [39], therefore, a balance is established between these two behaviors.

3.4. Analysis of factors affecting L-threonine production (ΔTHR)

ANOVA results are shown in Table 4, which suggest that the linear terms for Whey, RTVH and T, and the quadratic terms for Whey

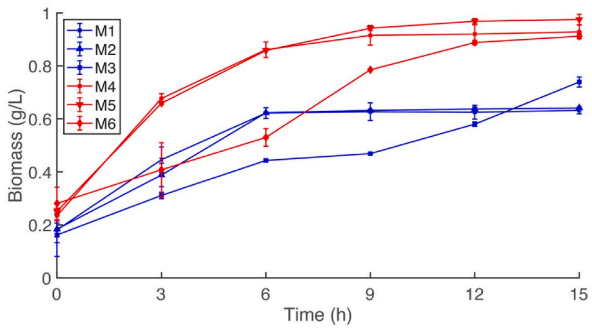


Fig. 1. Results of the first stage: effect of Whey and RTVH (lines in red) for biomass (X) by *E. coli.* GLC (M1), LAC (M2), Whey (M3). GLC + RTVH (M4), LAC + RTVH (M5) and Whey + RTVH (M6).

Table 4
ANOVA for biomass and L-threonine production by *E. coli*.

	ΔX		ΔTHR	
Source	F-value	p-value	F-value	p-value
Model	5.89	0.0035	6.26	0.0008
Whey			0.0569	0.8136
RTVH	4.16	0.0520	11.03	0.0030
T	7.42	0.0116	4.76	0.0396
$Whey^2$			4.50	0.0450
T^2	6.09	0.0208	12.76	0.0016
Lack of Fit	0.3340	0.9579	0.7662	0.6964
R^2	0.4142		0.5765	
Adjusted R^2	0.3439		0.4844	

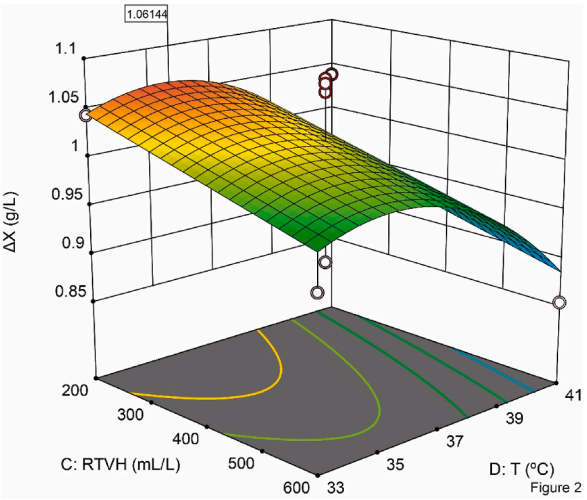


Fig. 2. Response Surface for biomass production by *E. coli*, showing the interaction between RTVH and T.

and T, must be considered. Although the adjusted regression coefficient is not so high (0.4844), the lack of fit is not significant and the model is statistically significant (p-value<0.05), then the model can be used in numerical optimization.

Equation (2) shows the model representing the effect of Whey, RTVH and T on ΔTHR, which can be considered accurate because it fulfills the assumptions of normality, homoscedasticity and independency of residuals [68].

$$\Delta THR = -7.51887 + 0.129937 * Whey - 0.000317 * RTVH + 0.405392 * T - 0.008541 * Whey^2 - 0.005619 * T^2 \quad (2)$$

Fig. 3 shows the response surfaces for ΔTHR as a function of Whey and RTVH content, Whey and T, T and RTVH content. It is possible to observe that ΔTHR is favored by Whey values closer to 8 g/L. In case of T, ΔTHR is favored between 35 °C and 37 °C. In the case of the RTVH, it can be observed that there is a negative relationship between it and ΔTHR, therefore higher values of RTVH content than 200 mL/L decrease the ΔTHR.

3.5. Numerical optimization

As already discussed, the results from the experimental designed allowed obtaining quadratic, statistically significant regression models (p-value<0.05) for predicting biomass and ΔTHR as a function of the studied factors. For the multi-objective numerical optimization, more importance was assigned to the ΔTHR (level 5 vs level 3 for ΔX). Results of the optimization suggested a total of 99 scenarios with a desirability higher than 0.8. From those, the scenario with content of 7.29 g/L of GLC, 8.16 g/L of LAC in whey, 200 mL/L of RTVH, and a T of 35.68 °C was the chosen. This scenario was validated in a new experimental run. On the other hand, the temperature identified, being 1.3 °C lower than that commonly reported for L-threonine production with *E. coli*, i.e. 37 °C [15,69,70], this result suggest that using Whey and RTVH might lead to energy savings in the bioprocess.

3.6. Experimental validation of the optimal

Experimental test using the obtained optimum (with the highest desirability) was carried out by triplicate in shake flasks, adjusting the $(NH_4)_2SO_4$ content for keeping the $R_{C/N} = 6.6$. The validation results shown in Table 5 compare the experimental values against the optimal predicted for the THR and biomass production by *E. coli*. As it can be observed, ΔTHR obtained experimentally is higher than

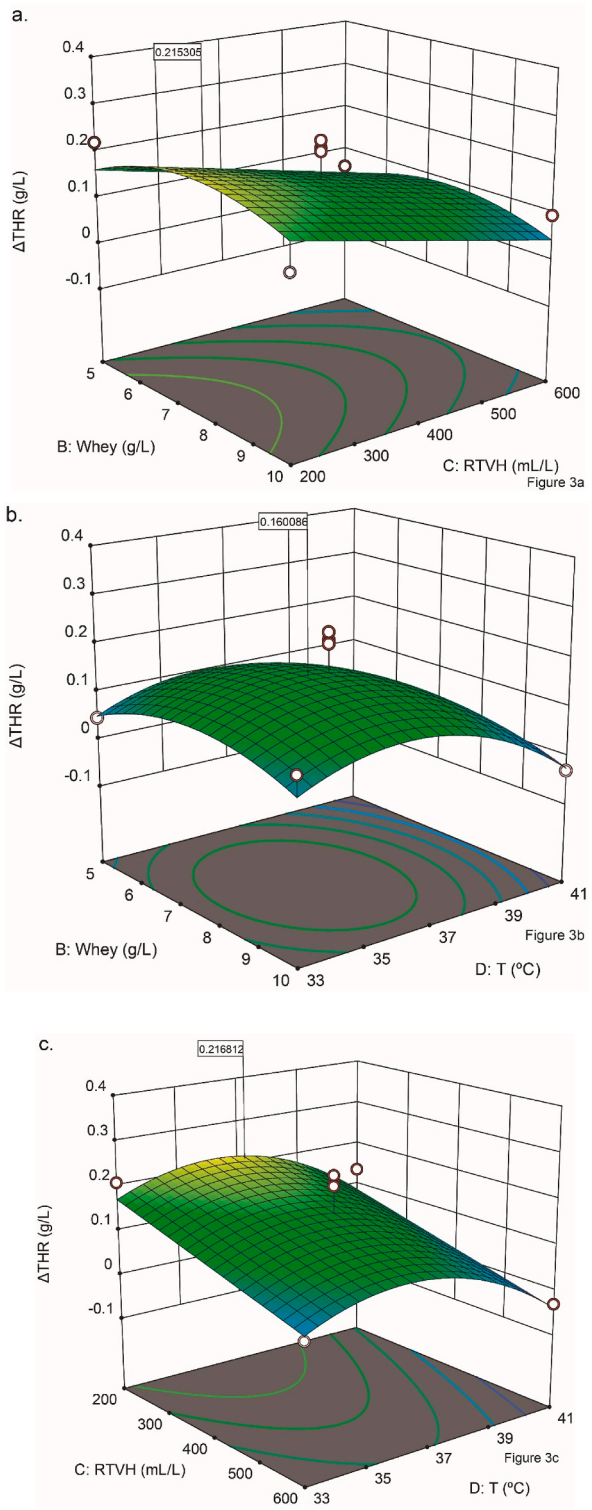


Fig. 3. Response surfaces for ΔTHR by *E. coli* showing the interaction between: **a.** Whey and RTVH; **b.** Whey and T; **c.** RTVH and T.

the optimal predicted, whereas ΔX was lower during the experimental validation runs, and also the yields were calculated $Y_{X/S} = 0.069$ and $Y_{P/S} = 0.036$. It is important to remark that the strain used in this study is a modified strain for improving L-threonine production, instead of biomass. Then, as THR is a secondary metabolite, its production is favored by growth deceleration. Furthermore, we have

Table 5
Optimal Predicted values vs. Experimental.

	ΔX (g/L)	ΔTHR (g/L)
Predicted	1.061	0.220
Experimental	0.767 ± 0.0402	0.406 ± 0.1223
Error	−27.7%	+84.5%

used inductors (methionine, isoleucine and proline) in the culture media, which might also explain why THR production is favored even at lower biomass (US3580810 patent [48]). It must also be noticed that during the multi-objective optimization, more importance was assigned to the THR (level 5) maximization regardless the resulting biomass (level 3).

A final mention must be done for the sake of a fair comparison between results obtained in this work and previously reported works on the production of THR. The amount of THR obtained in this work by using the *E. coli ATCC® 21277™* strain is lower than reported values by other authors [2,16,71], where genetically modified strains were used for improving THR production in chemically defined medium. In contrast, we have used a non-conventional substrate (a mixture of Whey and RTVH). To the authors' knowledge, the only published work on THR production using whey as substrate has reported a concentration of 3.6 g/L [33], there are recent reports of higher THR production but with chemically defined culture media and non-marketable *E. coli* strains [15,16]. After that, no reports have been published in the open literature addressing the THR production from complex substrates, by *E. coli*. Therefore, results shown in this work are a starting point in our research goal towards the development of a feasible process (from the technical and economical points of view), for THR production using agro-industrial wastes.

4. Conclusions

This work has demonstrated the technical feasibility of using whey and Red Tilapia (*Oreochromis* sp.) viscera hydrolysates for the production of L-threonine by *E. coli ATCC® 21277™*. In the first stage, the results showed that the use of Whey and Red Tilapia (*Oreochromis* sp.) viscera hydrolysates in the culture media enhance the growth of *E. coli*. In the second stage, results indicate that biomass production is affected by the hydrolysate content and process temperature. On the other hand, L-threonine production has shown to be affected by Whey and hydrolysate content, as well as by the temperature. In the third stage, it was possible to maximize L-threonine production, but with a detriment on biomass production. Finally, the complications associated to the use of these complex substrates (i.e., Whey and Red Tilapia (*Oreochromis* sp.) viscera hydrolysates) for the production of L-threonine by *E. coli*, require more in detail studies at the metabolic level, in order to understand and propose strategies to increase the process productivity, before its scale up.

Author contribution statement

Jhon Fredy Vélez Blandón: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Claudia Patricia Sánchez Henao, José Edgar Zapata Montoya: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Silvia Ochoa: Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thankfully recognize the support provided by the Universidad de Antioquia through the calls *"Thematic Research Projects 2020: Science and innovation in response to university and national challenges"* (project No. 2020–33174), and the "Programmatic Call: Engineering and Technology 2019–2020" (project No. 2020–33781).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18744.

References

[1] A. Kayser, J. Weber, V. Hecht, U. Rinas, Metabolic Flux Analysis of Escherichia coli in Glucose-Limited Continuous Culture . I . Growth-Rate- Dependent Metabolic Efficiency at Steady State, 2018, pp. 693–706, https://doi.org/10.1099/mic.0.27481-0.

[2] Y. Fang, J. Wang, W. Ma, J. Yang, H. Zhang, L. Zhao, S. Chen, S. Zhang, X. Hu, Y. Li, X. Wang, Rebalancing microbial carbon distribution for L-threonine maximization using a thermal switch system, Metab. Eng. 61 (2020) 33–46, https://doi.org/10.1016/j.ymben.2020.01.009.

[3] M.T. Kidd, B.J. Kerr, L-threonine for Poultry: a review, J. Appl. Poult. 2 (1996) 358–367.

[4] Y.W. Dong, L. Feng, W.D. Jiang, Y. Liu, P. Wu, J. Jiang, S.Y. Kuang, L. Tang, W.N. Tang, Y.A. Zhang, X.Q. Zhou, Dietary threonine deficiency depressed the disease resistance, immune and physical barriers in the gills of juvenile grass carp (Ctenopharyngodon idella) under infection of Flavobacterium columnare, Fish Shellfish Immunol. 72 (2018) 161–173, https://doi.org/10.1016/j.fsi.2017.10.048.

[5] A.V. Malinovsky, Reason for indispensability of threonine in humans and other mammals in comparative aspect, Biochem 82 (2017) 1055–1060, https://doi.org/10.1134/S0006297917090097.

[6] X. Dong, P.J. Quinn, X. Wang, Metabolic engineering of Escherichia coli and Corynebacterium glutamicum for the production of L-threonine, Biotechnol. Adv. 29 (2010) 11–23, https://doi.org/10.1016/j.biotechadv.2010.07.009.

[7] M.H. Lee, H.W. Lee, J.H. Park, J.O. Ahn, J.K. Jung, Y. Il Hwang, Improved l-threonine production of Escherichia coli mutant by optimization of culture conditions, J. Biosci. Bioeng. 101 (2006) 127–130, https://doi.org/10.1263/jbb.101.127.

[8] P.H. Janssen, Propanol as an end product of threonine fermentation, Arch. Microbiol. 182 (2004) 482–486, https://doi.org/10.1007/s00203-004-0732-y.

[9] R. Tao, Y. Jiang, F. Zhu, S. Yang, A one-pot system for production of l-2-aminobutyric acid from l-threonine by l-threonine deaminase and a NADH-regeneration system based on l-leucine dehydrogenase and formate dehydrogenase, Biotechnol. Lett. 36 (2014) 835–841, https://doi.org/10.1007/s10529-013-1424-y.

[10] V.A. Lukyanova, A.I. Druzhinina, S.M. Pimenova, V.A. Ioutsi, A.G. Buyanovskaya, R.U. Takazova, E.V. Sagadeev, A.A. Gimadeev, Thermodynamic properties of L-threonine, J. Chem. Thermodyn. 116 (2018) 248–252, https://doi.org/10.1016/j.jct.2017.09.022.

[11] M. Rieping, T. Hermann, L. Threonine, Amino Acid Biosynthesis, 2006, pp. 71–92, https://doi.org/10.1007/7171_2006_065.

[12] M. D'Este, M. Alvarado-Morales, I. Angelidaki, Amino acids production focusing on fermentation technologies – a review, Biotechnol. Adv. 36 (2018) 14–25, https://doi.org/10.1016/j.biotechadv.2017.09.001.

[13] Y. Li, D. Zhang, N. Cai, C. Han, Q. Mao, T. Wang, Q. Zhou, N. Chen, X. Xie, Betaine supplementation improved L-threonine fermentation of Escherichia coli THRD by upregulating zwf (glucose-6-phosphate dehydrogenase) expression, Electron. J. Biotechnol. 39 (2019) 67–73, https://doi.org/10.1016/j.ejbt.2019.03.004.

[14] Threonine Market: Global Industry Analysis and Forecasting 2027, 2021. https://www.maximizemarketresearch.com/market-report/global-threonine-market/98333/. (Accessed 7 October 2022).

[15] S. Wang, Y. Fang, Z. Wang, S. Zhang, L. Wang, Y. Guo, X. Wang, Improving l-threonine production in Escherichia coli by elimination of transporters ProP and ProVWX, Microb. Cell Factories 20 (2021), https://doi.org/10.1186/s12934-021-01546-x.

[16] L. Zhao, Y. Lu, J. Yang, Y. Fang, L. Zhu, Z. Ding, C. Wang, W. Ma, X. Hu, X. Wang, Expression regulation of multiple key genes to improve l-Threonine in Escherichia coli, Microb. Cell Factories 19 (2020) 1–23, https://doi.org/10.1186/s12934-020-01312-5.

[17] S.Y. Lee, J.H. Park, Integration of systems biology with bioprocess engineering: L-threonine production by systems metabolic engineering of Escherichia coli, Adv. Biochem. Eng. Biotechnol. 120 (2010) 1–19, https://doi.org/10.1007/10_2009_57.

[18] S. Liu, Y. Liang, Q. Liu, T. Tao, S. Lai, N. Chen, T. Wen, Development of a two-stage feeding strategy based on the kind and level of feeding nutrients for improving fed-batch production of l-threonine by Escherichia coli, Appl. Microbiol. Biotechnol. 97 (2013) 573–583, https://doi.org/10.1007/s00253-012-4317-4.

[19] C. Petit, Y. Kim, S.K. Lee, J. Brown, E. Larsen, D.R. Ronning, J.W. Suh, C.M. Kang, Reduction of feedback inhibition in homoserine Kinase (ThrB) of corynebacterium glutamicum enhances L-threonine biosynthesis, ACS Omega 3 (2018) 1178–1186, https://doi.org/10.1021/acsomega.7b01597.

[20] X. Dong, Y. Zhao, J. Hu, Y. Li, X. Wang, Attenuating L-lysine production by deletion of ddh and lysE and their effect on L-threonine and L-isoleucine production in Corynebacterium glutamicum, Enzym. Microb. Technol. 93–94 (2016) 70–78, https://doi.org/10.1016/j.enzmictec.2016.07.013.

[21] I. Shiio, Threonine production by dihydrodipicolinate synthase-defective mutants of Brevibacterium flavum, Biotechnol. Adv. 8 (1990) 97–103.

[22] V.G. Debabov, The Threonine Story, Adv. Biochem. Eng. Biotechnol., 2003, pp. 113–136.

[23] M. Ikeda, S. Takeno, Amino Acid Production by Corynebacterium Glutamicum, 2013, pp. 107–147, https://doi.org/10.1007/978-3-642-29857-8_4.

[24] L. Eggeling, H. Sahm, Amino acid production, in: Compr. Biotechnol., Elsevier, 2011, pp. 563–571, https://doi.org/10.1016/B978-0-444-64046-8.00186-5.

[25] Y.O. Motta-Correa, W.J. Mosquera, M., Aprovechamiento del lactosuero y sus componentes como materia prima en la industria de alimentos, Cienc. Tecnol. Aliment. 13 (2015) 81–91.

[26] R. Chourasia, L.C. Phukon, M.M. Abedin, S. Padhi, S.P. Singh, A.K. Rai, Whey valorization by microbial and enzymatic bioprocesses for the production of nutraceuticals and value-added products, Bioresour. Technol. Reports. 19 (2022), 101144, https://doi.org/10.1016/j.biteb.2022.101144.

[27] M.I. González Siso, The biotechnological utilization of cheese whey: a review, Bioresour. Technol. 57 (1996) 1–11, https://doi.org/10.1016/0960-8524(96)00036-3.

[28] J. Hausjell, M. Miltner, C. Herzig, A. Limbeck, Z. Saracevic, E. Saracevic, J. Weissensteiner, C. Molitor, H. Halbwirth, O. Spadiut, Valorisation of cheese whey as substrate and inducer for recombinant protein production in E. coli HMS174(DE3), Bioresour. Technol. Reports. 8 (2019), https://doi.org/10.1016/j.biteb.2019.100340.

[29] G. Zhang, H.M. Zabed, J. Yun, J. Yuan, Y. Zhang, Y. Wang, X. Qi, Two-stage biosynthesis of D-tagatose from milk whey powder by an engineered Escherichia coli strain expressing L-arabinose isomerase from Lactobacillus plantarum, Bioresour. Technol. 305 (2020), https://doi.org/10.1016/j.biortech.2020.123010.

[30] L.M. Rosales-Colunga, E. Razo-Flores, L.G. Ordoñez, F. Alatriste-Mondragón, A. De León-Rodríguez, Hydrogen production by Escherichia coli ΔhycA ΔlacI using cheese whey as substrate, Int. J. Hydrogen Energy 35 (2010) 491–499, https://doi.org/10.1016/j.ijhydene.2009.10.097.

[31] P.R. Ferreira Rosa, S.C. Santos, E.L. Silva, Different ratios of carbon sources in the fermentation of cheese whey and glucose as substrates for hydrogen and ethanol production in continuous reactors, Int. J. Hydrogen Energy 39 (2014) 1288–1296, https://doi.org/10.1016/j.ijhydene.2013.11.011.

[32] C. Fischer, T. Kleinschmidt, Synthesis of galactooligosaccharides using sweet and acid whey as a substrate, Int. Dairy J. 48 (2015) 15–22, https://doi.org/10.1016/j.idairyj.2015.01.003.

[33] Y.T. Ko, J.R. Chipley, Microbial production of lysine and threonine from whey permeate, Appl. Environ. Microbiol. 45 (1983) 610–615, https://doi.org/10.1128/aem.45.2.610-615.1983.

[34] D. Carranza-Saavedra, C.P. Sánchez Henao, J.E. Zapata Montoya, Kinetic analysis and modeling of L-valine production in fermentation batch from E. coli using glucose, lactose and whey as carbon sources, Biotechnol. Reports. 31 (2021), https://doi.org/10.1016/j.btre.2021.e00642.

[35] J. Ding, S. You, W. Ba, H. Zhang, H. Chang, W. Qi, R. Su, Z. He, Bifunctional utilization of whey powder as a substrate and inducer for β-farnesene production in an engineered Escherichia coli, Bioresour. Technol. 341 (2021), 125739, https://doi.org/10.1016/j.biortech.2021.125739.

[36] A. Bosso, A.C. Iglecias Setti, A.B. Tomal, S. Guemra, L.R.I. Morioka, H.H. Suguimoto, Substrate consumption and beta-galactosidase production by Saccharomyces fragilis IZ 275 grown in cheese whey as a function of cell growth rate, Biocatal. Agric. Biotechnol. 21 (2019) 1878–8181, https://doi.org/10.1016/j.bcab.2019.101335.

[37] E.A. Córdoba, J.C. Alonso González, D.C. Cuevas Tarazona, F. Núñez Dasilva, Como conservar y utilizar los desperdicios del pescado, 2017.

[38] A.E. Ghaly, V.V. Ramakrishnan, M.S. Brooks, S.M. Budge, D. Dave, Fish processing wastes as a potential source of proteins, amino acids and oils: a critical review, J. Microb. Biochem. Technol. 5 (2013) 107–129, https://doi.org/10.4172/1948-5948.1000110.

[39] R. Safari, A. Motamedzadegan, M. Ovissipour, J.M. Regenstein, A. Gildberg, B. Rasco, Use of hydrolysates from yellowfin tuna (Thunnus albacares) heads as a complex nitrogen source for lactic acid bacteria, Food Bioprocess Technol. 5 (2012) 73–79, https://doi.org/10.1007/s11947-009-0225-8.

[40] L.J.G. Sampedro, J.E.Z. Montoya, Effects of hydrolysis and digestion in Vitro on the activity of bovine plasma hydrolysates as inhibitors of the angiotensin i converting enzyme, Braz. Arch. Biol. Technol. 57 (2014) 386–393, https://doi.org/10.1590/S1516-89132014005000004.

[41] J.B.S. Andrea, O.D.B. Nelly, E.Z.M. José, Efecto de Temperatura, pH, Concentración de Sustrato y Tipo de Enzima en la Hidrólisis Enzimática de VÍsceras de Tilapia Roja (Oreochromis spp.), Inf. Tecnol. 27 (2016) 63–76, https://doi.org/10.4067/S0718-07642016000600007.
[42] L.J. Gómez, N.A. Gómez, J.E. Zapata, G. López-García, A. Cilla, A. Alegría, In-vitro antioxidant capacity and cytoprotective/cytotoxic effects upon Caco-2 cells of red tilapia (Oreochromis spp.) viscera hydrolysates, Food Res. Int. 120 (2019) 52–61, https://doi.org/10.1016/j.foodres.2019.02.029.
[43] P. Esakkiraj, G. Austin Jeba Dhas, A. Palavesam, G. Immanuel, Media preparation using tuna-processing wastes for improved lipase production by shrimp gut isolate staphylococcus epidermidis CMST Pi 2, Appl. Biochem. Biotechnol. 160 (2010) 1254–1265, https://doi.org/10.1007/s12010-009-8632-x.
[44] Y. Triki-Ellouz, B. Ghorbel, N. Souissi, S. Kammoun, M. Nasri, Biosynthesis of protease by Pseudomonas aeruginosa MN7 grown on fish substrate, World J. Microbiol. Biotechnol. 19 (2003) 41–45.
[45] V. Klompong, S. Benjakul, D. Kantachote, F. Shahidi, Use of protein hydrolysate from yellow stripe trevally (selaroides leptolepis) as microbial media, Food Bioprocess Technol. (2012) 1317–1327.
[46] I. Petrova, I. Tolstorebrov, I. Zhivlyantseva, T.M. Eikevik, Utilization of fish protein hydrolysates as peptones for microbiological culture medias, Food Biosci. 42 (2021), 101063, https://doi.org/10.1016/j.fbio.2021.101063.
[47] N. Chen, J. Huang, Z. Bin Feng, L. Yu, Q.Y. Xu, T.Y. Wen, Optimization of fermentation conditions for the biosynthesis of L-threonine by escherichia coli, Appl. Biochem. Biotechnol. 158 (2009) 595–604, https://doi.org/10.1007/s12010-008-8385-y.
[48] lsamu Shiio, Kamakura-shi, S. Nakamori, K. Sano, US3580810, US3580810, Fermentative production of L-threonine, 1971, https://doi.org/10.1145/178951.178972.
[49] S.A.F. Kusuma, I. Parwati, T. Rostinawati, M. Yusuf, M. Fadhlillah, R.R. Ahyudanari, Y. Rukayadi, T. Subroto, Optimization of culture conditions for Mpt64 synthetic gene expression in Escherichia coli BL21 (DE3) using surface response methodology, Heliyon 5 (2019), https://doi.org/10.1016/j.heliyon.2019.e02741.
[50] O.C. Amadi, E.J. Egong, T.N. Nwagu, G. Okpala, C.O. Onwosi, G.C. Chukwu, B.N. Okolo, R.C. Agu, A.N. Moneke, Process optimization for simultaneous production of cellulase, xylanase and ligninase by Saccharomyces cerevisiae SCPW 17 under solid state fermentation using Box-Behnken experimental design, Heliyon 6 (2020), https://doi.org/10.1016/j.heliyon.2020.e04566.
[51] A. Manzoor, J.I. Qazi, I.U. Haq, H. Mukhtar, A. Rasool, Significantly enhanced biomass production of a novel bio-therapeutic strain Lactobacillus plantarum (AS-14) by developing low cost media cultivation strategy, J. Biol. Eng. 11 (2017), https://doi.org/10.1186/s13036-017-0059-2.
[52] T. Katipoglu-Yazan, S. Dev, E.D.-L. Quéméner, T. Bouchez, Data on the influence of temperature on the growth of Escherichia coli in a minimal medium containing glucose as the sole carbon source for the joint computation of growth yields and rates at each temperature from 27 to 45°C, Data Brief 48 (2023), 109037, https://doi.org/10.1016/j.dib.2023.109037.
[53] R.L. Buchanan, L.A. Klawitter, The effect of incubation temperature, initial pH, and sodium chloride on the growth kinetics of Escherichia coli O157:H7, Food Microbiol. 9 (1992) 185–196, https://doi.org/10.1016/0740-0020(92)80046-7.
[54] R. Noor, Z. Islam, S.K. Munshi, F. Rahman, Influence of temperature on Escherichia coli growth in different culture media, J. Pure Appl. Microbiol. 7 (2013) 899–904.
[55] D. Kazan, A. Çamurdan, A. Hortaçsu, The effect of glucose concentration on the growth rate and some intracellular components of a recombinant E. coli culture, Process Biochem. 30 (1995) 269–273, https://doi.org/10.1016/0032-9592(95)85008-2.
[56] N.N. Deshavath, G. Mukherjee, V.V. Goud, V.D. Veeranki, C.V. Sastri, Pitfalls in the 3, 5-dinitrosalicylic acid (DNS) assay for the reducing sugars: interference of furfural and 5-hydroxymethylfurfural, Int. J. Biol. Macromol. 156 (2020) 180–185, https://doi.org/10.1016/j.ijbiomac.2020.04.045.
[57] G. Lorentz Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31 (1959) 426–428.
[58] L.J. Gómez Sampedro, Obtención de compuestos funcionales mediante hidrólisis enzimática de proteínas de vísceras de tilapia roja (Orechromis sp.), Universidad de Antioquia, 2019. https://www.bps.go.id/dynamictable/2018/05/18/1337/persentase-panjang-jalan-tol-yang-beroperasi-menurut-operatornya-2014.html.
[59] C.T. Sepúlveda Rincón, Evaluation of a Spray-Dried Enzymatic Hydrolysate of Red tilapia (Oreochromis spp.) Viscera as a Source of Bioactive Peptides, Universidad de Antioquia, 2020.
[60] S.I. Aspmo, S.J. Horn, V.G.H. Eijsink, Hydrolysates from Atlantic cod (Gadus morhua L.) viscera as components of microbial growth media, Process Biochem. 40 (2005) 3714–3722, https://doi.org/10.1016/j.procbio.2005.05.004.
[61] R. Bradstreet, The Kjeldahl Method for Organic Nitrogen, New York, 1965, https://doi.org/10.1016/c2013-0-12545-7.
[62] J.W. Henderson, R.D. Ricker, B.A. Bidlingmeyer, C. Woodward, Rapid , accurate , sensitive , and reproducible HPLC analysis of amino acids, Amino Acids (2000) 1–10. www.agilent.com/chem (accessed April 18, 2021).
[63] X. Hua, D. Fan, Y. Luo, X. Zhang, H. Shi, Y. Mi, X. Ma, L. Shang, G. Zhao, Kinetics of high cell density fed-batch culture of recombinant escherichia coli producing human-like collagen, Chin. J. Chem. Eng. 14 (2006) 242–247, https://doi.org/10.1016/S1004-9541(06)60065-7.
[64] J. Zhu, K. Shimizu, Effect of a single-gene knockout on the metabolic regulation in Escherichia coli for D-lactate production under microaerobic condition, Metab. Eng. 7 (2005) 104–115, https://doi.org/10.1016/j.ymben.2004.10.004.
[65] W. Mendenhall, R.J. Beaver, B.M. Beaver, Introducción a la probabilidad y estadística, Decimo ter, Cengage Learning, 2010. http://latinoamerica.cengage.com.
[66] M.H. Kutner, C. Nachtsheim, J. Neter, W. Li, Applied Linear Statistical Models, McGraw-Hill Irwin, Fourth, 2004.
[67] Y.H. Lim, H.L. Foo, T.C. Loh, R. Mohamad, R. Abdul Rahim, Z. Idrus, Optimized medium via statistical approach enhanced threonine production by Pediococcus pentosaceus TL-3 isolated from Malaysian food, Microb. Cell Factories 18 (2019), https://doi.org/10.1186/s12934-019-1173-2.
[68] O. Melo, L.A. López, S. Melo, Diseño de Experimentos Métodos y Aplicaciones, Universidad Nacional de Colombia Facultad de Ciencias, 2020, https://doi.org/10.36385/FCBOG-4-0.
[69] D. Gong, J. Qiao, H. Li, Y. Li, D. Huang, Z. Wang, X. Hu, X. Wang, Deleting chaperone-usher fimbriae operons to improve L-threonine production in Escherichia coli, Syst. Microbiol. Biomanufacturing. 1 (2023) 3, https://doi.org/10.1007/s43393-023-00166-3.
[70] T. V Vybornaya, T. V Yuzbashev, A.S. Fedorov, D.M. Bubnov, S.S. Filippova, F. V Bondarenko, S.P. Sineoky, Use of an alternative pathway for isoleucine synthesis in threonine-producing strains of Escherichia coli, Appl. Biochem. Microbiol. 56 (2020) 759–769, https://doi.org/10.1134/S0003683820070066.
[71] J. Yang, Y. Fang, J. Wang, C. Wang, L. Zhao, X. Wang, Deletion of regulator-encoding genes fadR, fabR and iclR to increase L-threonine production in Escherichia coli, Appl. Microbiol. Biotechnol. 103 (2019) 4549–4564, https://doi.org/10.1007/s00253-019-09818-8.