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ORIGINAL ARTICLE

Production L-lactic acid from different starch sources by optimization media Composition using fermenter 2Liter Scale

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ABSTRACT
The current research was designed for improve production of L-lactic acid by optimized three Lactic acid bacteria (LAB) group through enhancement of the production medium. (LAB) consist from (Lactobacillus bulgaricus, Lactobacillus paracasei, Lactobacillus heltivicus) and using three type of medium. All three Strains could produce lactic acid in these media but at different production amounts. To acquire the maximum L-lactic acid production from inexpensive medium, some ingredients (corn, rice straw, Sweet Potato as a main carbon sources, pH)for L-lactic acid screening medium and L-lactic acid production conditions were inspected to obtain the optimum concentrations. These isolates provided the concentration of lactic acid ranging from 5.95-1.24, 4.51-1.65and 2.25-1.52(g/L) and total acidity expressed ranging from 1.42-1.30, 1.33 -0.83, and 1.00-0.33% in the three medium respectively All the results derived from this research have been found to be Optimization medium for developed L-lactic acid production .
Keywords:*starch, corn, rice straw, Sweet Potato, fermenter.*

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INTRODUCTION

Lactic acid Considered is one of significant organic acids comprehensive diversity for modern applications. Lactic acid (C3H6O3) is one of the least complicated normal carboxylic acid [1]. This acid is considered as one of the key intermediate of numerous manufactures comprehensive wood, detergent and food industries [2]. Furthermore, two optical isomers from lactic acid: D (−)-lactic acid and L (+)-lactic acid. DL-lactic acid is produced by chemical composition from petrochemical resources, an optically pure L (+) or D (−)-lactic acid can be obtained by fermentation microbial of renewable resources when the suitable microorganism that can produce only one of the isomers selected [3]. Also lactic acid very important source for producing poly lactic acid polymer (PLA) which is a main, alternate material for lactic acid bio-production, there are a great source of carbon is needed as substrate [4]. For the bioprocess product for lactic acid to be effective, inexpensive raw materials are essential, polymer producers and other processing users generally require great quantities of lactic acid at a comparatively minimum cost [5]. Raw materials for lactic acid production have to the following features: low scales in contaminants, rapid production rate, high yield, cheap, little or no by-product formation, capability to ferment with a few or no pretreatment. [6].Newly, the demand for lactic acid has been growing progressively, in particularly the request was evaluated to be 300,000–450,000 tons per year worldwide [7]. The inexpensive raw materials, such as starch and cellulosic materials, applied for lactic acid production [8] starchy and cellulosic materials are presently a major deal of concern, because they are inexpensive, renewable and superabundant [9].The starchy materials applied for lactic acid production involve, potato, corn, rice, wheat, barley and cassava [10]. These materials able to hydrolyze to fermentable sugars prior fermentation process, Sweet potato, Corn and rice are important industrial crops for starch production in many countries [11].Furthermore, transferring the fermentation technology to other starchy products would contribute to add values and diversify the use of raw materials [12]. The main objective of this work were estimate the effect of L-lactic acid fermentation by different starch type derived from corn, sweet potato and rice by using optimize three types of lactic acid bacteria(LAB)in Bioreactor 2 Liter Scale.

MATERIAL AND METHODS

Medium Optimization

For L-lactic acid medium optimization experiments, Lactic acid bacteria (LAB) were cultivated on the three media which composed of (g/L): in **Table 1:**

Media	Components .Formula
Medium 1	Glucose 10.00, Peptone 10.00 ,Meat extract 8.00 ,Yeast extract 4.00 $MgSO_4$.7H2O 0.20, MnSO4.H2O 0.05, agar 10.00.
Medium 2	Glucose 10.00 , Meat extract 8.00,Yeast extract 4.00 , K_2HPO_4 6.00 ,Tri-Ammonium citrate 1.00 , MgSO4.7H2O 0.57, $MnSO_4.4H_2O$ 0.12 ,$FeSO_4.7H_2O$ 0.03 .
Medium 3	Glucose 10.00, Peptone 5.00, Yeast extract10.00g, K_2HPO_4 1.50, Sodium acetate 1.50, $MnSO_4.4H_2O$ 0.2, $MgSO_4.7H_2O$ 0.05, agar 10.00.

Final pH 7.0 at 25°C

Method Selection for L-lactic acid-produce isolate

Strains isolates were selected for L-lactic acid detection using High-performance liquid chromatography (HPLC) via a comparison of retention times of the standard lactic acid (*Lactobacillus bulgaricus, Lactobacillus paracasei, Lactobacillu sheltivicus*). Were able to produce L-lactic acid (Table.1), (Table.2), (Table.3). When cultivated in the three media at 35°C for 48 h under anaerobic conditions. The degree of acidity was measured by Titration method and Optical purity by next equation D-lactic acid = (1-(L-lactic acid / total lactic acid)) × 100.This isolates were chosen for beginning search.

Table 2:lactic acid productions by 3 Strains selected using medium No: 1 incubation at 35°C for 48 h under anaerobic conditions.

Strains	pH	Total acidity (%)	Lactic acid concentration (g/L)		Optical purity (%)	
			L-Lactic acid	D-Lactic acid	L-Lactic acid	D-Lactic acid
Lactobacillus bulgaricus	3.55	1.42	5.95	2.50	60.08	39.92
Lactobacillus paracasei	4.35	1.30	2.79	1.24	63.07	36.93
Lactobacillus heltivicus	3.38	1.17	3.58	1.46	86.54	13.46

Table 3:lactic acid productions by 3 Strains selected using medium No: 2incubation at 35°C for 48 h under anaerobic conditions.

Strains	pH	Total acidity (%)	Lactic acid concentration (g/L)		Optical purity (%)	
			L-Lactic acid	D-Lactic acid	L-Lactic acid	D-Lactic acid
Lactobacillus bulgaricus	3.45	1.33	4.51	1.80	72.36	27.64
Lactobacillus paracasei	3.66	0.83	2.73	1.65	63.33	36.67
Lactobacillus heltivicus	3.79	1.00	3.05	1.74	63.68	36.32

Table 4:lactic acid productions by 3 Strains selected using medium No: 3incubation at 35°C for 48 h under anaerobic conditions.

Strains	pH	Total acidity (%)	Lactic acid concentration (g/L)		Optical purity (%)	
			L-Lactic acid	D-Lactic acid	L-Lactic acid	D-Lactic acid
Lactobacillus bulgaricus	3.18	1.00	2.25	1.90	65.30	34.70
Lactobacillus paracasei	3.79	0.40	1.30	1.59	52.22	47.78
Lactobacillus heltivicus	3.48	0.33	2.15	1.52	55.56	44.44

Optimization of several Conditions for L-lactic acid production

To acquire the maximum production of L-lactic acid from cheap medium, several ingredients (corn starch, rice straw starch, Sweet Potato starch as a major carbon sources) of L-lactic acid screening medium and the L-lactic acid production conditions were investigated to obtain the optimum concentrations.

Effect of different rice straw starch concentrations on L-lactic acid production in different media

The suitable concentration of rice straw starch for L-lactic acid production by the selected chosen (*Lactobacillus bulgaricus*) was specified using Medium: 1, 2, and 3 containing 0, 2, 4, 6, 8, and 10 (g/L) from rice straw starch. The results showed that the L-lactic acid concentration increased with the increase in rice straw starch concentration up to 10 (g/L) in media No: 1. the maximum L-lactic acid 4.40(g/L) was acquired at 48 h fermentation with an elementary rice straw starch concentration. While the maximum L-lactic acid in media No: 2 reached to 3.35(g/L) and 2.00(g/L) in same concentration of rice straw optimized. The L-Lactic acid concentration reduce when rice straw starch was higher than 8 (g/L).

Table 5:L-lactic acid production after cultivation in different media containing various concentrations of rice straw starch at 35°C for 48 h.

rice straw starch (g/l)	pH[a]			L-Lactic acid concentration (g/l)[b]			total sugars (g/l)[c]		
	M[1]	M[2]	M[3]	M[1]	M[2]	M[3]	M[1]	M[2]	M[3]
0	7.00	7.00	7.00	0.44	0.15	0.20	2.00	3.50	3.40
2	6.55	6.00	6.80	1.00	0.45	0.90	3.45	3.40	3.00
4	5.90	5.50	5.00	1.50	0.90	1.40	3.50	3.00	3.10
6	5.00	4.70	5.10	3.20	1.30	1.80	4.55	4.10	3.80
8	4.80	4.76	4.80	3.80	2.80	2.20	5.10	5.20	4.20
10	4.50	4.40	4.20	4.40	3.35	2.00	5.00	5.50	4.40

M * = medium No, a = pH Prop. b =HPLC analysis. c= Colorimetric (phenol-sulphuric acid) method.

Effect of different corn starch concentrations on L-lactic acid production in different media

The convenient concentration of corn starch for L-lactic acid production by the chosen isolate (*Lactobacillus bulgaricus*) was specific using Medium: 1, 2, and 3 containing 0, 2, 4, 6, 8, and 10 (g/L) from corn starch. The outcomes showed that the L-lactic acid concentration increased with the increase in corn starch concentration up to 8.00 (g/L) in media No: 1. the maximum L-lactic acid 5.50(g/L) was acquired at 48 h fermentation with an elementary corn starch concentration. While the maximum L-lactic acid in media No: 2 reached to 4.00 (g/L) and 2.50 (g/L) in same concentration of corn starch optimized. The L-Lactic acid concentration decreased when corn starch was higher than 8 (g/L). This may due to suppression by raise substrate concentration. Therefore, 8 (g/L) of corn starch concentration was chosen to be applied as the cheap carbon source in media No: 1 for L-lactic acid production by the isolate (*Lactobacillus bulgaricus*).

Table 6:L-lactic acid production after cultivation in different media containing various concentrations of corn starch at 35°C for 48 h.

corn starch (g/l)	pH[a]			L-Lactic acid concentration (g/l)[b]			total sugars (g/l)[c]		
	M[1]	M[2]	M[3]	M[1]	M[2]	M[3]	M[1]	M[2]	M[3]
0	7.00	7.00	7.00	0.60	0.35	0.20	4.00	4.30	3.00
2	5.15	5.00	4.80	2.85	0.85	0.80	4.15	4.40	3.00
4	4.80	5.10	5.00	4.50	1.30	1.90	5.50	5.00	2.10
6	4.81	4.70	4.70	5.10	2.50	2.10	6.25	5.25	1.80
8	4.60	4.66	4.00	5.50	4.00	2.50	7.30	5.50	1.55
10	4.66	4.40	4.00	5.46	3.20	2.00	7.00	5.50	1.00

M * = medium No, a = pH Prop. b =HPLC analysis. c= Colorimetric (phenol-sulphuric acid) method.

Effect of different Sweet Potato starch concentrations on L-lactic acid production in different media

The appropriate concentration of Sweet Potato starch for L-lactic acid production by the chosen isolate (*Lactobacillus bulgaricus*) was specific utilizes Medium: 1, 2, and 3 containing 0, 2, 4, 6, 8, and 10 (g/L) from Sweet Potato starch. The results showed that the L-lactic acid concentration increased with the increase in Sweet Potato starch concentration up to 8 (g/L) in media No: 1. the maximum L-lactic acid 7.50(g/L) was acquired at 48 h fermentation with an elementary Sweet Potato starch concentration. While the maximum L-lactic acid in media No: 2 reached to 3.40(g/L) and 3.20 (g/L) in same concentration of Sweet Potato starch optimized. The L-Lactic acid concentration reduce when Sweet Potato starch was higher than 8 (g/L).

Table 7:L-lactic acid production after cultivation in different media containing various concentrations of Sweet Potato starch at 35°C for 48 h.

Sweet Potato starch (g/l)	pH[a]			L-Lactic acid concentration (g/l)[b]			total sugars (g/l)[c]		
	M[1]	M[2]	M[3]	M[1]	M[2]	M[3]	M[1]	M[2]	M[3]
0	7.00	7.00	7.00	0.85	0.35	0.40	5.10	4.60	4.20
2	6.75	6.70	6.80	2.80	1.50	1.70	5.15	5.30	5.00
4	5.90	5.95	5.88	4.80	2.20	1.90	5.40	5.00	5.10
6	5.81	5.70	5.75	5.60	2.55	2.10	5.20	5.30	5.10
8	4.90	4.96	4.95	7.50	3.40	3.20	6.00	5.80	5.55
10	4.76	4.70	4.60	7.00	3.20	3.00	6.10	6.00	5.70

M * = medium No, a = pH Prop. b =HPLC analysis. c= Colorimetric (phenol-sulphuric acid) method.

Therefore, and through the previous results obtained from this research It is clear that Sweet Potato starch Better than rice straw starch and corn starch by examining them in the three media and according to the sequential results 7.50 (g/L), 4.00 (g/L) and 5.5 (g/L) for production L-lactic acid by *Lactobacillus bulgaricus* from different starchas a main carbon source.

L- Lactic acid production and Kinetics of cell growth in batch cultivation fermenter working volume 2 litter

For the major scale fermentation process Sweet Potato starch which is cheap and abundantly obtainable raw material when compared to glucose, sucrose and lactose. The expense of analytical degree sugar such as glucose was around double higher than that of corn starch. Thus, 8 g/l of Sweet Potato starch concentration was selected to be utilized as the cheap carbon source in Medium: 1 for L-lactic acid production by the isolate *Lactobacillus bulgaricus*. The growth of *Lactobacillus bulgaricus* was evaluated in bioreactor working volume 2 litters using the optimized medium. The bioreactor was run under uncontrolled pH conditions, where the pH was initially set at 7.2. Figure: 1 showed that the pH of the medium drastically dropped to about 4.85 after 24 hours and 3.90 after 48 hours of cultivation.The cell mass increased gradually and reached its maximum 19.00 [$g.L^{-1}$] after time cultivation. for batch bioreactor 2 litter cultivation without controlled pH The Total reducing sugar start decreasing faster from 14.50[$g.L^{-1}$] to 0.00[$g.L^{-1}$] in end of cultivation, also it is clear the total reducing sugar decreases inversely proportional to the increased production of L-lactic acid .The specific growth rate of *Lactobacillus bulgaricus* was 0.050 [h^{-1}] more than controlled pH cultivation and the accumulated L-lactic acid reached

16.25 [g.L^{-1}] after 48h of cultivation with a production rate of almost 0.350 [g.L^{-1}.h^{-1}]. While Under uncontrolled pH cultivation, the dissolved oxygen level decreased to 75% after 24 hours Cultivation.

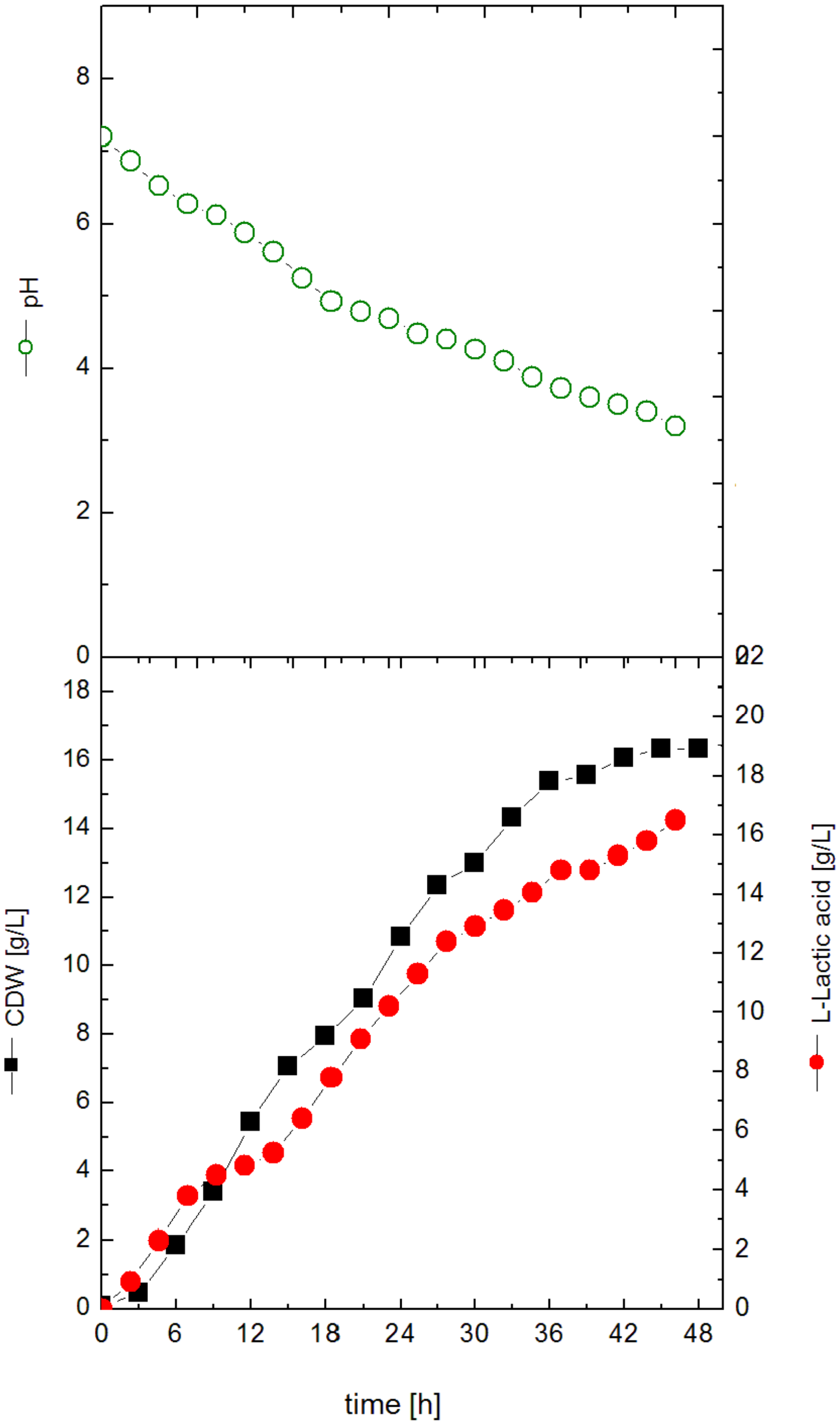

Fig: 1 L-Lactic acid production, cell dry weight and pH changes in batch fermenter 2-L cultivation with un-controlled pH.

CONCLUSION

In synopsis, kinetics data for whole experiments proceed in this research are briefed in fig: 1 mentioned above. All starchy materials, corn, rice straw, Sweet Potato as a main carbon source It had the ability to produce L-lactic acid, But with uneven productivity The best of them were chosen for shake flasks stage and 2-L fermenter scale . On parallel these outcome data as a conclusion on scaling up the process from shake flask to fermenter scale, uncontrolled pH fermenter produced more L-lactic acid production which is 16.25 [g.L-1]. For growth rate production, uncontrolled pH fermenter produces more which is reach to

0.363 [g.L-1.h-1]. Shake flask tests for determination of the optimum conditions for the fermentation process. Moreover, the batch cultivation in 2-L fermenter under un-controlled pH conditions showed important improvement in terms of L-lactic acid production.

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