STUDY OF THE FERMENTATIVE CAPACITY AND ETHANOL PRODUCTION OF TWO MICROORGANISMS ISOLATED FROM BOVINE RUMEN

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Abstract: The aim of this work was to determinate the fermentation conditions for two wild yeast isolated from bovine rumen. A factorial design 3x3x2 evaluating temperature (35, 40, and 45° C), agitation (0, 100, and 200 rpm), and strain type (LR2 and LR4) was used. The analyzed responses were growth and fermentation parameters. Results showed that the best growth conditions were 35° C and 200 rpm for the development of the strains LR2 and LR4. On the other hand, the best results obtained in alcoholic fermentation were observed at the temperature of 45° C with no agitation for the strain LR2. The thermal stability of the strain LR2 may have a viable application in industrial fermentative processes in tropical climates.

Keywords: Fermentation • Yeast • Bioethanol.

Introduction: The ethanol production as an energetic alternative has been the mayor interest subject from the beginning of the oil crisis in the 70's. As a result, the necessity to produce ethanol with high yields using low cost materials and energy consumption has taken interest in recent years. In order to solve this problematic, several methodologies have been proposed. The use of microorganisms with the capacity to produce high concentration of ethanol or to metabolize 5 and/or 6 carbon sugars, the use of mixed cultures to increase sugars consumption and ethanol yields are some of the strategies reported (Tao et al., 2005). Some of the microorganisms capable to metabolize carbohydrates have been isolated from soil, decaying vegetable materials, industrial effluents, municipal wastes, manure, and rumen. However, their fermentative ability is related to their isolation environment (Arellano et al., 2008; Ten et al., 2004). This fermentative ability may also be affected by other factors such as temperature and agitation. Changes in temperature and/or agitation may negatively affect the process and as a result low yields or absence of the interest metabolite are observed (Arellano et al., 2008). The aim of this work was to determinate the fermentative conditions in synthetic medium for two wild yeast isolated from bovine rumen.

Materials and methods: Two wild yeasts (LR2 and LR4) isolated from fistulated bovine rumen from Yucatan, Mexico and in process of identification were used in this study. The factorial design 3x3x2 with 18 treatments is showed in Table 1. The evaluated factors were temperature (35, 40, and 45° C), agitation (0, 100, and 200 rpm), and strain (LR2 and LR4). A seed culture for each strain was carried out in a nine-milliliter test tube for 24 h containing YPD medium (20 g/L glucose, 10 g/L yeast extract, and 20 g/L peptone of casein) and pH of 4.5 units. Temperature and agitation were set as mentioned in Table 1 for each essay. Seed cultures were inoculated in 250 mL Erlenmeyer flasks containing 90 mL of YPD medium. Cultures lasted 24 hours and kinetics parameters were obtained every 2 hours. Total microbial population was determined by direct method using a microscope (objective 40x) and a Neubauer chamber. Biomass quantification was obtained by dry weight method. The Dinitrosalicylic acid (DNS) method established by Miller (1959) was used to quantify the free reducing sugars. Ethanol

concentration was quantified by the technique of potassium dichromate of Bohringer and Jacob (1964). Maximal growth rate (μ_{max}), doubling time (td), and biomass yield (Yx/s) were the growth kinetic parameters estimated and the ethanol production kinetic parameters were product yield (Yp/s), maximal productivity (P_{max}), and fermentation efficiency. All results were statistically analyzed using the software Statgraphics Centurion XV.I.

Table 1. Factorial design 3x3x2.

	Encoded factors			Non-coded factors		
Treatments	A	B	C	Temperature (° C)	Agitation (rpm)	Strains
1	-	-	-	35	0	LR2
2	0	-	-	40	0	LR2
3	+	-	-	45	0	LR2
4	-	0	-	35	100	LR2
5	0	0	-	40	100	LR2
6	+	0	-	45	100	LR2
7	-	+	-	35	200	LR2
8	0	+	-	40	200	LR2
9	+	+	-	45	200	LR2
10	-	-	+	35	0	LR4
11	0	-	+	40	0	LR4
12	+	-	+	45	0	LR4
13	-	0	+	35	100	LR4
14	0	0	+	40	100	LR4
15	+	0	+	45	100	LR4
16	-	+	+	35	200	LR4
17	0	+	+	40	200	LR4
18	+	+	+	45	200	LR4

Results and discussion: Kinetic results of growth and alcohol production of every treatment are listed in Table 2. Highest values of dry weight and biomass yield were observed in treatment 16 and 7 (35° C and 200 rpm) with the strains LR4 and LR2 respectively (Table 2). For the alcohol production, the highest values of product yield, fermentation efficiency and alcohol production efficiency were observed in treatment 8 with the strain LR2 (40° C and 200 rpm) followed by the results obtained with the same strain in treatment 3 (45° C and 0 rpm).

Data were analyzed using ANOVA and the results are summarized in Table 3. These results indicate that temperature ant agitation had a significant effect in all the analyzed responses. They also suggest that the strain used had a significant effect in the ethanol production and doubling time. The interaction between temperature and agitation had a direct effect to all responses and the interaction of the three factors (temperature, agitation, and strain) had a significant effect to the ethanol production and doubling time.

In order to explain these effects, Figure 1(a) represents that the temperature, agitation, and their interaction have an effect statistically significant in the dry weight response (biomass). Contrary to that, there was no effect when the strain was changed. Figure 1(b) presents the fermentation efficiency between the strains tested. This chart exhibits a statistically difference between the strains, LR2 had better results than LR4.

Table 2. Kinetic results for growth and production for the strains LR2 and LR4.

Treatments	Max. Pop. ($x10^6$ el/mL)	Dry weight (g/L)	μ_{max} (h^{-1})	Td (h)	$Y_{x/s}$	Ethanol (g/L)	$Y_{p/s}$	Efficiency (%)	P_{max} (g/Lh)
1	179	1.87	0.34	2.10	0.10	7.94	0.43	84.30	0.79
2	129	1.73	0.27	2.53	0.09	7.53	0.38	74.50	0.63
3	227	1.53	0.26	2.63	0.08	8.70	0.45	88.24	0.54
4	293	2.80	0.60	1.15	0.15	8.20	0.43	84.30	1.03
5	248	2.83	0.38	1.81	0.15	7.99	0.42	82.40	0.80
6	272	2.70	0.58	1.20	0.14	8.04	0.43	84.30	0.67
7	439	4.22	0.57	1.22	0.22	7.62	0.40	78.40	0.76
8	124	3.90	0.45	1.55	0.20	9.40	0.50	98.03	0.94
9	0	0	0	0	0	0	0	0	0
10	53	1.70	0.34	2.04	0.09	8.00	0.42	82.40	0.80
11	31	1.70	0.41	1.70	0.09	7.40	0.39	76.50	0.62
12	0	0	0	0	0	0	0	0	0
13	130	2.99	0.69	1.00	0.16	7.62	0.42	82.40	1.10
14	95	2.14	0.41	1.70	0.11	6.88	0.36	70.59	0.57
15	105	3.30	0.55	1.25	0.18	5.90	0.32	62.70	0.42
16	219	4.72	0.55	1.26	0.25	7.80	0.41	80.40	0.78
17	142	3.12	0.43	1.63	0.17	6.20	0.33	64.70	0.52
18	0	0	0	0	0	0	0	0	0

Table 3. Results from ANOVA

	Dry weight (g/L)	μ_{max} (h^{-1})	Td (h)	$Y_{x/s}$	Ethanol (g/L)	Efficiency (%)	P_{max} (g/Lh)
Temperature (°C) - **A**	*	*	*	*	*	*	*
Agitation (rpm) - **B**	*	*	*	*	*	*	*
Strains - **C**			*		*	*	*
AB	*	*	*	*	*	*	*
AC			*	*	*	*	*
BC			*		*	*	
ABC			*		*	*	*

* Statistically significant difference at 95% confidence

Table 4. Most suitable conditions obtained from LSD test at 95%

Stage	Temperature (°C)	Agitation (rpm)	Strains
Growth	35	200	LR2, LR4
Alcoholic fermentation	45	0	LR2

During the fermentation, both strains showed similar results during their development (biomass concentration); however for the ethanol production the strain LR2 exhibited better results.

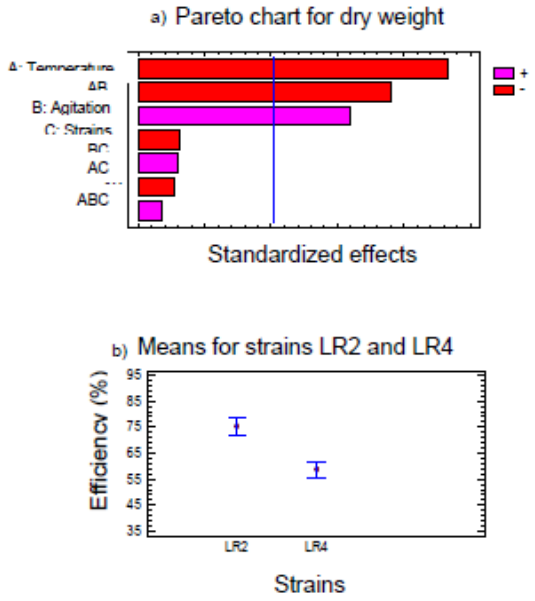

Figure 1. Pareto chart and mean for answers: dry weight and efficiency of alcoholic fermentation.

According to the obtained results and the statistical analysis, it is possible to suggest that the best fermentation conditions (growth and ethanol production) for the strains testes in this experiment are showed in Table 4.

Conclusions: The wild strains isolated from bovine rumen showed the ability to metabolize glucose to produce ethanol. These strains are statistically different concerning the ethanol production, however in the growth parameters they seem to be similar. Temperature and agitation were observed to have a significant effect in all the analyzed responses. Under the conditions of 45° C and no agitation the results obtained with the strain LR2 were the best for the ethanol production.

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References:

Arellano M, Pelayo C, Ramírez J, Rodríguez I 2008. Characterization of kinetic parameters and the formation of volatile compounds during the tequila fermentation by wild yeasts isolated from agave juice. J Ind. Microbiol. Biotechnol 35: 835–841.

Bohringer P, Jacob L 1964. The determination of alcohol using chromic acid.Zeitschr. Flussinges Abst. 31: 223.

Miller G 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar.Analytical Chemistry. 31(3): 426-428.

Tao F, Miao J, Shi G, Zhang K 2005. Ethanol fermentation by an acid-tolerant *Zymomonas mobilis* under non-sterilized condition. Process Biochemistry 40: 183-187.

Ten LN, Im W-T, Kim M-K, Kang MS, Lee S-T 2004.Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. J Microb Methods 56: 375:-382.