Maestría en Ingeniería en Diseño de Bioprocesos

Title

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Culture of *Aspergillus oryzae* and *Saccharomyces cerevisiae* on whey to produce single cell protein for cattle feeding

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1. Introduction

Whey is the liquid obtained from the production of cheese when the coagulated protein is separated after straining. According to data from INEGI, in Puebla the production of whey is approximately 449 000 L/yr, which means a large production of potentially contaminating material. Main places where whey is produced in the state are St. Gregorio Atzompa, Atlixco, Ocoyucan, Huejotzingo, St. Andrés Cholula, Huaquechula and St. Martín Texmelucan [1].

The nutritional capacity of whey is used to cultivate microorganisms such as *Aspergillus oryzae* and *Saccharomyces cerevisiae*, which have probiotic properties for cattle.

Currently, the process most widely used to produce biomass is using ultrafiltration; however, it is a high cost technique; for this reason the use of whole whey is a better option to obtain single cell protein at lower cost, either through axenic, mixed or staged culture.

2. Objectives

2.1. General objective

To produce single cell protein (SCP) from cultures on whey of *A. oryzae* and *S. cerevisiae* through different fermentative systems to be used at cattle feeding.

2.2. Specific objectives

- To characterize the whey through proximal analysis.
- To carry out whey cultures of *A. oryzae* and *S. cerevisiae*, single and mixted.
- To implement a staged culture system for both microorganisms.
- To characterize the SCP obtained by fermentation, using as a reference the Mexican Standards.

3. Methodology

1. Characterization of whey

- Protein
- Lactose
- Fat
- Total Solids
- Ash
- pH
- Moisture
- Lactic acid
- Chlorides

2. Treatments

- pH
- Temperature
- Yeast extract

3. Fermentation

- Axenic cultures
- Mixed culture
- Two-Stage culture

4. Characterization of SCP

- Total protein
- Non-proteic nitrogen
- Raw protein
- Total Nitrogen
- True Protein
- Fat
- Carbohydrates
- Ash
- Moisture
- Crude Fiber
- Ether Extract
- Nucleic acids
- Chlorides

Figure 1. Methodology used in this research

2. Treatments

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- Temperature
- Yeast extract

3. Fermentation

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- Mixed culture
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Figure 1. Methodology used in this research

Table 1. Factorial design where X1 is temperature (° C), X2, pH and X3, addition of yeast extract (g/L)

Treatment	X_1:T	X_2:pH	X_3:YE
1	+(120)	+(7)	+(10)
2	+(120)	+(7)	-(0)
3	+(120)	-(4)	-(0)
4	+(120)	-(4)	+(10)
5	-(60)	+(7)	-(0)
6	-(60)	+(7)	+(10)
7	-(60)	-(4)	+(10)
8	-(60)	-(4)	-(0)

4. Results

The chemical composition of the whey from St. María Tonantzintla is reported at Table 2, where the data is compared with other similar results [2], [3].

Table 2. Chemical composition comparison of whey

Parameter	Composition (%)	Miranda *et al* (2009)	Hernández (2013)
Lactose	3.63	4.1	4.1
Protein	0.90	0.94	2.54
pH	4.70	4.22	4.77
Lactic Acid	0.87	0.32	0.33
Chlorides	0.32	---	---
Moisture	92.56	---	94.79
Total Solids	7.60	6.4	5.21
Ash	0.47	---	0.49

After 72 hours since the inoculation of both microorganisms, growth is observed in all flasks, but significant biomass production is observed at pH=7.

Figure 2. Growth in flask at pH=7 and sterilized on top and growth at pH=4 and pasteurized on bottom.

The results from ANOVA are shown at Figure 5 for *Saccharomyces cerevisiae* and Figure 6 for *Aspergillus oryzae*. Both graphs show the interaction between factors and their impact over biomass production in order to define the best conditions for the cultures.

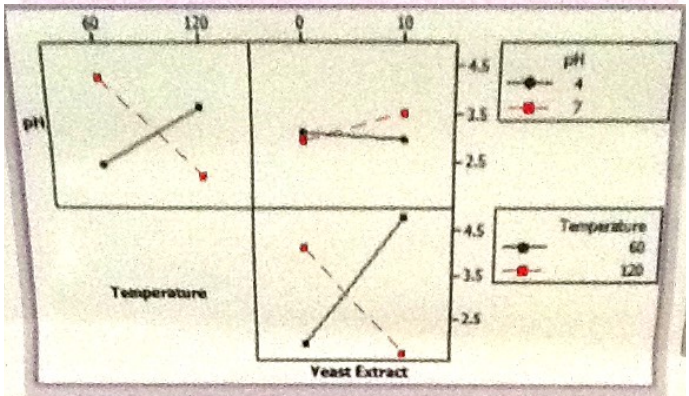

Figure 3. Effect of the interaction T-pH, T-YE and pH-YE over S. cerevisiae biomass.

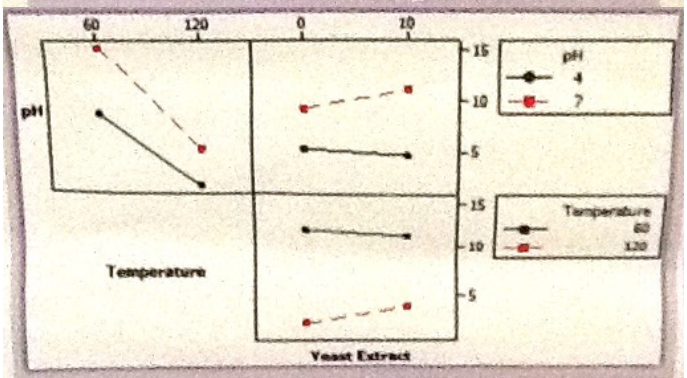

Figure 4. Effect of the interaction T-pH, T-YE and pH-YE over A. oryzae biomass

In the case of *S. cerevisiae* the interaction between pH-T and T-YE are determinant for biomass production being pH=7, 60 ° C and 10 g/L of yeast extract the best conditions. On the other hand, for *A. oryzae* cultures, the most important factors are pH and temperature but their interaction or another are not. Although the addition of yeast extract could increase the biomass production, the change is small; therefore, the best conditions for this culture are pH=7, 60 ° C and without yeast extract.

Once the best cultivation conditions were known, the axenic cultures of the microorganisms were carried out, obtaining the following growth and substrate consumption kinetics that can be observed at Figures 7 and 8.

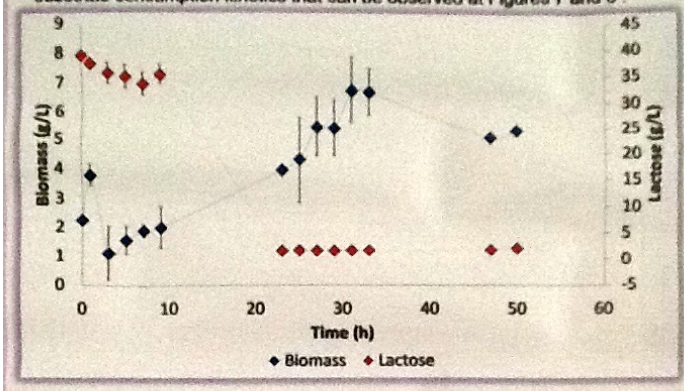

Figure 5. Growth and substrate consumption kinetics for Saccharomyces cerevisiae

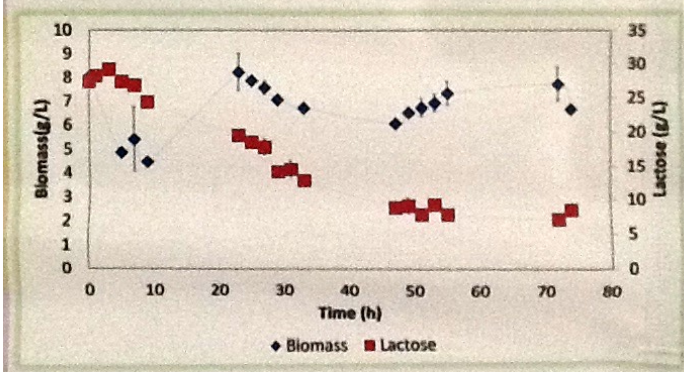

Figure 6. Growth and substrate consumption kinetics for Aspergillus oryzae

Table 3. Performance of A. oryzae and S. cerevisiae cultures

Microorganism	Xmax	Rx	Rs	Yxs
A. oryzae	8.25 g/L	0.267 g/Lh	1.35 g/Lh	0.197 gx/gs
S. cerevisiae	6.75 g/L	0.201 g/L	1.211 g/Lh	0.166 gx/gs

Finally, a two-stage culture is proposed in which *A. oryzae* will be growth on a first stage, after the medium pass through an internal metallic mesh and an external glass fiber filter; in the second stage, *S. cerevisiae* will be growth.

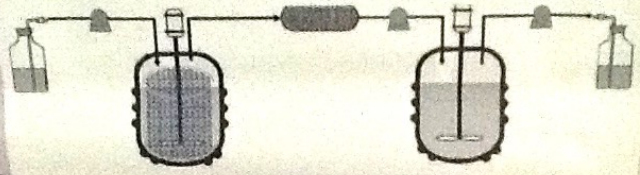

Figure 7. Diagram of two-stage culture

5. Conclusions

- According to the results from ANOVA, the best treatment is 60 ° C, pH=7 and 10 g/L of yeast extract for *S. cerevisiae*. In the same way, 60 ° C, pH=7 and without of yeast extract for *A. oryzae*.
- Both microorganisms consumed the sugars contained in the whey and produce levels of biomass similar to treatments in flask. However, the medium is heterogeneous and to determine biomass production is hard because of insoluble protein is an interference in all direct techniques. Later, indirect techniques must be used to eliminate the error.

References

[1] INEGI. Censo Agropecuario: Cría y explotación de animales en Puebla. México: Instituto Nacional de Estadística y Geografía, 2007.

[2] Miranda, O., Ponce, I., Fonseca, P.L., Cutiño, M., Díaz, R.M. and Cedeño, C., "Características físico-químicas de sueros de queso dulce y ácido producidos en el combinado de quesos de Bayamo", Rev Cub Aliment Nutr, vol. 19, pp. 21-25, 2009.

[3] Hernández, R., "Caracterización fisicoquímica de un producto tipo cajeta elaborado a partir del suero dulce de quesería", Facultad de Ciencias Químicas, Universidad Veracruzana, Reporte de Experiencia Recepcional, México, 2013.

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