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Key Words

Microbial biomass protein; Distillery slops decontamination; Yeast production; Research & development cycle; Fodder protein; Rich fodder feedstocks

Abbreviation

COD: Chemical Oxygen Demand; MGP: Microbial Growth Promoters; MGE: Microbial Growth Enhancer

Research Article

A Bioethanol Waste Challenge being a Source of High-Quality Protein-Rich Feedstock: A New Technology

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Abstract

Microbial biomass protein from Candida utilis yeast was produced using distillery vinasse as the sole source of carbon and energy. The process was carried out from lab scale to 200 m3 fermentors. Propagation media have to be supplemented with nutrients since vinasse cannot provide all compounds needed for a stable operation. Different mixtures of molasses-enriched vinasse were tested and finally, a Microbial Growth Enhancer (MGE) was incorporated into the media to ensure its optimization. MGE-supplemented vinasse at 0.03 kgm3 was found optimum for yeast propagation. At an industrial scale, μmax ≥ 0.33 h-1 was obtained which allowed a stable behavior of the culture at this scale and significant industrial productivity. Yeast biomass from vinasse showed a composition and amino acid profile very similar to those previously obtained from molasses medium. Nutritional tetestsemonstrated that vinasse´s yeast is an excellent source of proteins for animal feeding and an efficient pollution reducer.

Introduction

The Cuban Institute for Research on Sugarcane By-Products (ICIDCA) exhibits a vast experience of more than 40 years in the field of yeast technology. This journey set off with the early lab research works about the development of Candida utilis (fodder yeast) from sugacane molasses. The results were implemented at industrial scale in 1963 when a 30t/d factory started. Later from 1979 to 1982, ten new plants were installed and operated at full scale. ICIDCA's technicians, researchers and engineers took part in those ventures defining the technology, doing equipment selection, strain evaluation, assembling and training of operating personnel among other tasks.

Sugarcane agro-industry is one of the most impact contaminating sources of underground waters [1]. However, on the other hand, it has been one of the most job-generating industries in Latin-American region and sugarcane ranges among the cultivars with the highest utilization efficiency of solar energy and its conversion into biomass. In ethanol production, wastewaters are composed by cooling waters from condenser and fermentation vats, as well as, liquid wastes from distillation. Among the wastewaters generated by sugar-ethanol industrial complexes, vinasses stand out as highly polluting agents due to their contents of organic compounds, whether biodegradable or not. Vinasses are produced at a rate of 10 to 16 cubic meters by cubic meters of distilled ethanol. Chemical Oxygen Demand (COD) of vinasses varies according to the fermentation and distillation efficiencies but roughly ranges from 30 to 65 kgm-3 [2].

The first lab approaches to the utilization of vinasses for the propagation of microbiol biomass can be tracked back to the late 60s [3]. The reduction of organic load and at the same time the production of valuable and scarce protein is the best feature of this process [4]. Yeast propagation from vinasses can be carried out at in continuous culture at lab scale for long periods of time [3]. However, when scaled up to industrial level, vinasses need the nutritional supplementation of the culture broth because otherwise the system stability would be extremely uncertain due to the poverty of this waste in essential nutrients. In addition, the typical fluctuations in industry would be an evident drawback as well. However, those inconveniences can be overcoming by supplementing them with industrial syrups from sugarcane industry, e.g., blackstrap molasses, B molasses, etc. or by a growth promoter available in the international market [5,6]. In respect to the technological feasibility of this process, three factories -based on this technology - operate in Cuba since 1999, with a production of more than 50 thousand tons up to now [2,7,4]. The present paper is an overview of the whole process development that made possible that achievement.

Materials and Methods

Microorganisms

Different yeasts from the specie Candida utilis were evaluated about their affinity to the carbon source. Among them, Candida utilis 129 from ICIDCA collection and several strains obtained from Pasteur Institute in France (C. utilis NRRL Y-660, NRRL Y-900, NRRL Y-1084 and NRRL Y-1082). Inoculums were prepared from agar-malt slants, grown overnight in a medium containing sugarcane molasses at 20 mg/ml of total reducing sugars concentration and nutrient salts (diammonium phosphate and sulphate) to cover cell nutritional requirements and grown in a rotary shaker at 32 ºC and pH 4.5. A 2.5 l Marubishi MD5 fermenter was used to start batch propagation with a medium composed by molasses-based slops from a local distillery at a COD concentration of 60 mg/ml supplemented with above mentioned salts in such a way that in all cases COD was the limiting factor of the substrate.

Chemical analysis

Nitrogen was determined according to Kjeldahl using a Kjeltec Auto System from Tecator AB, Sweden [8]. Reducing sugars were estimated by copper reduction according to [9]. Dry matter was done by desiccation at 105 ºC overnight until constant weight in a vacuum oven at 60 ºC. Ashes were determined by incineration at 600 ºC for 4 hours and referred to dry matter content.

Results and Discussion

Evaluation of yeast strains

Candida utilis strains were evaluated in a medium containing vinasse as the sole source of carbon and energy. All strains are capable to duplicate at a growth rate close to 0.300 h-1, with slight differences. It has been previously demonstrated [5], that can metabolize ethanol and glycerol present in the vinasses at a very similar rate. Those values were quite similar to ones reported here (Table 1) shows the values obtained for several kinetic parameters.

Table 1: Kinetic parameters of Candida utilis grown on vinasse as the sole source of carbon and energy. Media were supplemented with molasses (70:30 on COD basis).

	Strain 129	NRRL Y-660	NRRL Y-900	NRRL Y-1082	NRRL Y-1084
μmax, h^{-1}	0.328	0.341	0.335	0.33	0.3
Ks, $mgml^{-1}$	0.355	0.336	0.337	0.458	0.462
Yx/s	0.286	0.312	0.259	0.28	0.234
m, $mgml^{-1}$	0.109	0.102	0.119	0.123	0.126

The constant Ks shows, in all cases, relatively high values. It means, that despite yeasts can thrive on vinasses medium this environment is far for being optimal, since Ks represents the affinity of cells for the growing medium. Even when the culture was supplemented with molasses, growth parameters did not significantly improve (Table 2).

Table 2: Effect of nutrient supplement on vinasse-yeast Candida utilis behavior.

Vinasse-Molasses*	μmax, h^{-1}	Yx/s	COD Reduction, %
70:30:00	0.359	0.382	38.91
80:20:00	0.341	0.323	39.97
85:15:00	0.338	0.325	41.9
QZ-350, 0.03 $mgml^{-1}$	0.304	0.307	65.32
QZ-350, 0.05 $mgml^{-1}$	0.303	0.315	64.28

*expressed as COD

Vinasse-molasses mixtures lead to μmax values for C. utilis from 0.338 h^{-1} for 85:15 mixture to 0,359-1 at 70:30). Both growth rate and yields increased as molasses amount increased. However, from environmental point of view, lower amounts of molasses are preferred. Lower COD values in the propagation medium improved the overall organic load reduction. The nutrient contribution of molasses can be substituted by the addition of Microbial Growth Promoters (MGP). The use of MGP QZ-350 (Quimizuk, Havana), from local market, as a molasses substitute was studied first at the lab scale and then taken up to the full industrial operation. In both scales, excellent stability was achieved in continuous culture. From these results the three operating vinasse–yeast factories adopted the technology, using MGP QZ-350 instead of molasses (Figure 1) [5], shows the behavior of C. utilis in a medium composed of vinasse as the sole source of carbon and energy.

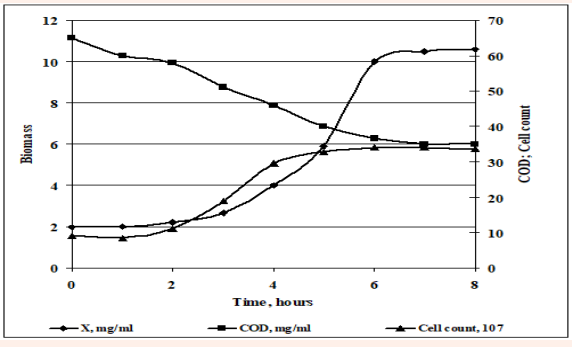

Figure 1: Multiplication and growth curves of Candida utilis NRRL Y-660 in batch culture on vinasse as unique source of carbon and energy.

Yeast propagation was tested at an industrial scale in a 220 m3 Vogelbusch fermentor. The culture was scaled from lab to full volume following the traditional steps in the factory. All of them were tested for: contamination with wild yeasts or bacteria, also cell morphology, cell concentration, and protein content were monitored. The behavior of the cell population in all steps was identical to that obtained for molasses yeast in similar conditions, (Figure 2) shows the kinetic pattern of yeast cells from lab to full scale [2].

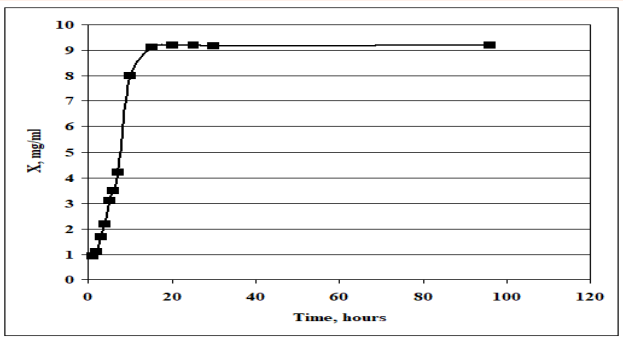

Figure 2: Behavior of yeast population during continuous culture at industrial scale. Operation conditions: temperature 35 °C, pH 3.8, D (=μ) = 0.33 h^{-1}.

The whole culture at the industrial level was stable for more than 90 hours. In fact, the same culture was maintained in the fomenters for the subsequent 4 months with no significant contamination, no cell degradation, and a protein content of about 45 %.

Conclusions

As shown in the paper a feasible industrially applied technology, that converts bioethanol highly polluting wastes from a headache into an economically attractive product of high demand, was demonstrated at the industrial commercial level. This result gives the possibility to engineer a productive scheme of a biorefinery, that, for the first time, ensures an eco-friendly production of energy and food in a highly effective way.

References

1. Pawar NJ, Pondhe GM, Patil SF (1998) Groundwater pollution due to sugar-mill effluent, at Sonai, Maharashtra, India. Environmental Geology 34(2-3): 151-158.
2. Otero MA, Saura G, Valdés I, Peña MA, Martínez J, et al. (2002) Operational analysis of the distillery-yeast plant complex at CAI Antonio Guiteras Part II ICIDCA. About derivatives 36(2): 7-10.
3. Almazán OA (1968) Use of residual musts for the production of forage yeast (torula) ICIDCA. On the derivatives 2(2): 17-26.
4. Otero MA, Saura G, Martínez JA, Almazán OA (2007) Fodder yeast production: a new approach for distillery vinasse treatment. Proc Int Soc Sugar Cane Technol 26: 1127-1133.
5. Martínez JA, Almazán OA, Saura G, Otero MA (2004) Production of fodder yeast from stillage: an environmental approach. Zuckerindustrie 129(2): 92-95.
6. Otero MA, Martinez JA, Saura G (2010) Propagation of Candida utilis from ethanol stillage and its individual components. About the derivatives 44(3): 27-32.
7. Saura G, Valdés IF, Martínez JA, Reyes E, Pascual A, et al. (2002) Yeast production technology using distillery stillage as the main source of carbon and energy. About the derivatives 36(1): 20-23.
8. Anon (1983) Nitrogen determination by Kjeltec Auto System, Application Note. 53, Tecator AB.
9. ICUMSA Method GS 4/3-3 (2007) Reducing Sugars in Cane Molasses and Refined Syrups by the Lane+Eynon Procedure.