FLOCCULENT YEAST POPULATION DYNAMICS IN A CONTINUOUS TOWER REACTOR FOR ETHANOL FUEL PRODUCTION

By

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Abstract

The purpose of this work was to follow the population dynamics of 6 yeast strains in a tower reactor for continuous ethanol production. The strains studied have grown flocculently and shown a fermentation performance appropriate to industrial use. The two glass reactors used were connected in line and fed with a sucrose based synthetic medium. For 15 days, the yeast populations were quantified through colony count over a differential medium (WLN). In such medium, the 6 strains tested were differentiated for presenting colonies of morphological patterns distinct from one another. The results have shown that there was a selection pressure within the reactors, since only two (Y5 and Y6) of the six inoculated strains were found in the process at the end of the trial. It is clear that when this type of unit is projected for industrial use, in addition to using a flocculent strain with suitable fermentation performance, it is equally necessary that a careful study of the raw material be used and the conditions in which one intends to act.

Introduction

The sugar and alcohol sector is extremely important for the Brazilian economy. It is estimated that 1 million direct jobs and 3 to 5 million indirect ones are created (Carvalho, 1999). Part of this importance results from the installation, in 1975, of the greatest biofuel program ever implemented, called ProÁlcool (Lima, 1975). Nowadays, this program, existing only in Brazil and in the United States (Wheals, 1999) has not been a priority for the Brazilian government, even though the annual ethanol production is still significant. In the 96–97 harvest season, Brazil produced 14.16 billion litres of ethanol and 13.8 million cubic metres of sugar. These products were produced by 328 industries in activity with 101 autonomous ethanol plants producing only ethanol, and 227 sugar mills producing sugar and ethanol (Zanin et al., 2000).

Simultaneously with the government’s alcohol policy, research centres have started working with new ethanol production processes. New continuous fermentation concepts have been developed, placing Brazil as the holder of this technology. Some mills (Guarani Mill, Alvorada Mill, Costa Pinto Mill) have installed a continuous fermentation process that has been optimised using mathematical modeling (Andrietta and Maugeri, 1994). In addition to presenting high yields (10 mL of ethanol/L of juice/h), this process also has more stability and flexibility as well as a lower input consumption.

A continuous fermentation process using a flocculent strain of S. cerevisiae has been installed in some industrial units. This process (NATRONTEC) is operating in at least 7 distilleries (Zanin et al., 2000).

According to these authors, the process presents great advantages when compared to conventional ones, especially regarding the elimination of separating machines, accounting for a significant share of the final cost of the product. The replacement of the flocculent yeast, used as an inoculum, for a native one is a great challenge for this type of process, since the juice can carry levels of $10^4$ yeasts/mL (Souza et al., 1993).

HOECHTS/UHDE has developed an alcohol fermentation process using its own flocculent yeast strain. The process was installed at one Distillery (Destilaria Nova Avanhadava—Avanhadava—SP—Brazil) in the 87–88 season. The process failure was due to the replacement of the original strain for a wild one. After some modifications, in the 95–96 harvest season, the installations designed to operate with a flocculent strain began to operate with a conventional process, in which a non flocculent S. cerevisiae was used as a starter. Forty days after the harvest season had started, an installation of flocculent strains was detected, probably originated from cane juice. Viegas (1999) has worked with 15 different flocculent strains isolated from this process and selected one of them, based on the sedimentation coefficient, to develop a fermentation system with fluidised bed. The optimised system has been able to work with a 93% yield and a 15.48 g/L.h. (ethanol) productivity.

This paper aims at evaluating the population dynamics of 6 flocculent yeast strains submitted to a continuous fermentation process for ethanol production in a tower reactor.

KEYWORDS: Flocculent Yeast, Ethanol Production, Continuous Fermentation.
Materials and methods

Yeast strains: six different yeast strains with flocculation characteristics have been used. These strains have been isolated from ethanol fermentation processes (Y1 and Y3—Destilaria Diana—Avanhadava—SP—1995 harvest season; Y2—Usina Costa Pinto—Piracicaba—SP—1998 harvest season; Y4—Usina Junqueira—Igarapava—SP—2000 harvest season; and Y5 and Y6—Usina Costa Pinto—Piracicaba—SP—1999 harvest season) and have shown an appropriate fermentative performance for industrial processes. Such performance was evaluated according to the fermentative potential technique (Andrietta et al. 1995) based on the determination of the kinetic parameter (maximum specific growth speed) and specific production parameters (ethanol and cellular mass). The specific productions are obtained in relation to the sugar consumed. The specific production values are compared to the ones reached by a reference strain (fermentative potential equals to 0), which has an average behavior among the ones used in Brazilian distilleries.

A WLN agar medium (DIFCO 0424) was used for the distinction among the strains. The parameters adopted in the differentiation of the colonies include: size, color profile, Specific Sedimentation Speed. The strains were cultivated for 24 hours at 32°C and 150 r/min, in 250 mL Erlenmeyer flasks containing 100 mL of synthetic medium: (g/L): glucose, 150; KH2PO4, 5.0; NH4Cl, 1.0; MgSO4, 7H2O, 1.0; KCl, 1.0; yeast extract, 6.0. 40 mL samples of the fermented wine were centrifuged at 4000 r/min for 5 minutes. The precipitated cell mass was re-suspended in sterile water and re-centrifuged twice. One gram of the final precipitate was diluted in 100 mL of water and after intense agitation the optic density (600 nm) was followed for 10 minutes at 30-second intervals.

The specific sedimentation speed (SSS) is shown in the equation:

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SSS = \frac{1 - \text{Abs}_t/\text{Abs}_0}{t_0 - t}
\]

Where: Abs 0—absorbance value measured at the start time t0

Abs—absorbance value measured at time t

Fermentation media (g/L): Sucrose, 150; KH2PO4, 5.0; NH4Cl, 5.0; MgSO4, 7H2O, 1.0; KCl, 1.0; yeast extract, 6.0.

Bioreactor: Two glass tower batch reactors with 2.0 litre capacity and in line have been used (0.058 m diameter and 0.78 m height). The operation temperature was kept at 32°C by using a water-recycling bath. A peristaltic pump fed the substrate. The reactors had exits for air/CO2 exhaustion, the fermented wine and sample withdrawal.

Inoculum: The 6 strains from PDA (Potato dextrose agar) were grown separately in a shaker for 24 h, at 32°C and 150 r/min in 250-mL Erlenmeyer flasks with 100 mL of fermentation media. Four flasks were prepared for each of the strains tested. The yeasts were mixed into a single flask for obtaining the inoculum.

Operation: The inoculum was added to the first reactor with a peristaltic pump. Then, the medium feed was started at a 10 mL/minute flow. Thus, the inoculum was distributed along the 2 reactors. Samples were taken from the two fermentors at 1, 2, 3, 4, 6, 8, 11, 13 and 15 days. The analyses performed were: ethanol and yeast count. A sample of the inoculum was taken before starting the reactor feed in order to quantify each of the yeasts introduced.

Analyses: Yeast count: the samples taken from two fermentors were submitted to dilution in line. 10^4 to 10^7 dilutions were labeled on the surface in WLN medium. The strain differentiation was made on the basis of morphological differentiation of colonies in the medium used. This differentiation comprised size and colour profile.

Results and discussion

The results show (Figures 1 and 2) that, after 24 hours of trial, strains 2, 3 and 4 were present in both fermentors, at the lowest concentration evaluated (<10^5).

In fermentor 1 there was initial predominance of strain 5. After the sixth day, a predominance of strain 6 was observed, a phenomenon that lasted until the end of the trial (15 days). The same trend was repeated for the second fermentor.

Contrary to what was expected, it was not possible to associate the sedimentation speed parameter to the dominance of strain 6 in the process. Strain 4 was expected to be the dominant one, since it presented the highest sedimentation speed (0.26/min). However, strain 6, with a low sedimentation speed (0.0015), was dominant in the process. The values obtained for the other strains were 0.1564, 0.1434, 0.0010 and 0.1482 for strains 1, 2, 3 and 5, respectively.

An important point to be considered is the test used for flocculation evaluation (sedimentation speed). Even though all strains are flocculent, a difference in the flocculation pattern has been observed. Such differences were observed when the yeasts grew isolated for inoculum obtainment. The cells in strain 6, though flocculated, have kept longer in suspension. Therefore, the sedimentation speed test may not have provided elucidating data in the study in question.

As for the fermentative performance of the strains used in the fermentors, the results obtained for fermentative potential provide enough data for stating that all strains have the necessary features to remain in the system. The values obtained for fermentative potential (specific ethanol production and cellular mass) and the maximum specific growth speed for all strains coincide with the ones that Andrietta et al. (1995) consider within the averages obtained for strains used in industrial processes. These values were 94.9 and 0.57/h; 300.0 and 0.51; 112.4 and 0.49; 72.2 and 0.50; 13.8 and 0.52; and 300.0 and 0.51 for strains 1, 2, 3, 4, 5 and 6 respectively.

It is possible to state that, based on this study, there has been selection pressure inside the reactor, since all strains, for being good fermentors and
flocculent, could have remained in the process at first. However, a conclusion has not been reached as to what factor has led to the said predominance.

As it has been previously reported, for the development of a reactor with fluidised bed for industrial use, with several factors acting as pressure factors, it is necessary, in addition to project conception studies, to conduct studies considering the type of yeast intended to use and the type of raw material to be processed.

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REFERENCES


DYNAMIQUE DES POPULATIONS DE LEVURE FLOCONNEUSE DANS UN REACTEUR TUBULAIRE CONTINU POUR LA PRODUCTION DE CARBURANT ETHYLIQUE

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Résumé
Cette étude avait pour but d’étudier la dynamique des populations de 6 lignées de levure dans un réacteur tubulaire pour une production continue d’éthanol. Les lignées étudiées se sont développées par floculation et ont démontré une performance de fermentation appropriée à l’usage industriel. Les deux réacteurs de verre utilisés furent connectés en ligne et alimentés par un milieu synthétique à base de saccharose. Durant quinze jours, les populations de levure furent quantifiées par comptage sur milieu différentiel (WLN). Dans ce milieu, les six lignées étudiées furent différenciées par les caractères différents que chaque culture présentait. Les résultats ont démontré qu’il y avait une pression de sélection au niveau des réacteurs, puisque seulement deux (Y5 et Y6) des six lignées inoculées furent retrouvées à la fin de l’essai. Il est clair que lorsque ce type d’appareil est utilisé sur une base industrielle, il faut non seulement ajouter une lignée floconneuse ayant une performance de fermentation adéquate, mais aussi étudier soigneusement la matière première utilisée ainsi que les conditions d’utilisation prévues.

Mots clés: levure floconneuse, production d’éthanol, fermentation continue.

DINÁMICA DE LA POBLACIÓN DE LEVADURA FLOCULANTE EN UN REACTOR DE TORRE CONTINUO PARA LA PRODUCCIÓN DE ETANOL CARBURANTE

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Resumen
El propósito de este trabajo fue seguir la dinámica de población de seis cepas de levadura en un reactor de torre para la producción continua de etanol. Las cepas estudiadas crecieron floculantemente y mostraron un comportamiento fermentativo apropiado para su empleo industrial. Los dos reactores de vidrio empleados fueron conectados en línea y alimentados con un medio sintético en base a sacarosa. La población de levaduras fue controlada durante 15 días mediante el conteo de colonias sobre medio diferencial (VVLN). En este medio, las 6 cepas estudiadas fueron diferenciadas mediante la observación de colonias con patrones morfológicos diferentes unos de otros. Los resultados mostraron que hubo una presión de selección dentro del reactor, ya que sólo dos (Y5 e Y6) de las 6 cepas inoculadas se encontraron en el proceso al final de las pruebas. Resulta claro que cuando este tipo de unidad se proyecta para uso industrial, además de emplear una cepa floculante con un conveniente comportamiento fermentativo, resulta igualmente necesario un estudio cuidadoso de las materias primas a emplear y de las condiciones en las cuales se intenta operar.