STUDY ABOUT THE INFLUENCE OF THE INOCUL TYPE UPON THE INVERTASE POTENTIAL OF BAKER YEASTS

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Abstract
For the submerged cultures the inoculum quality and quantity play an important part in obtaining different products of metabolism at high speeds and biosynthesis yields. Because the inoculum quality is well established, these studies haven't had in view the influence of inoculum quantity but quality on output capacity of invertase.

Resume
Pour les cultures submergées la qualité et la quantité de l'inoculum joue un rôle important pour obtenir des différents produits de métabolisme á haute vitesse et rendement de la biosynthèse. Lorsque la quantité de l'inoculum est bien établie ces études n'ont pas eu en vue l'influence de la quantité mais de la qualité de l'inoculum sur le rendement de la sucrose.

Rezumat
Pentru culturile submerse calitatea şi cantitatea inoculului joacă un rol important în obţinerea diverşilor produşi de metabolism la viteze mari şi randamente ridicate ale biosintezei. Deoarece cantitatea de inocul este bine stabilită, aceste studii nu au avut în vedere influenţa cantităşii ci a calităţii inoculului asupra randamentului invertazic

Introduction

The main invertase producers are yeasts *Saccharomyces cerevisiae*, cultivated on fermentative medium, which contain different sources of carbon, nitrogen, growth factors and minerals. Also, for the submerged cultures the inoculum quality and quantity play an important part in obtaining different products of metabolism at high speeds and biosynthesis yields.

Because the inoculum quality is well established, suggesting that concentration ~ 10^6 cells/cm^3 should be provided by inoculation these studies haven't had in view the influence of inocul quantity but quality on the invertase potential of baker yeasts (*Saccharomyces cerevisiae*).

It is necessary to cultivate microorganisms in the so called synchronous cultures, for the biosynthesis methods in view of obtaining compounds of practical importance and eliminated batch culture disadvantages. These cultures consist of same age cells and/or dimension and therefore they divide simultaneously (Zarnea, 1984).

The process of synchronous division lasts only for few generations, because the individual differences determine diphassing processes in the multiplying rhythm even between the descendants of a single cell.

Depending on the applied techniques, there are two types of cultures:

- Synchronous cultures – where cells have been selected of similar age and dimension from the beginning;
- Synchronized cultures – where cells were brought at the same division stage by certain physical and chemical treatment (Bahrim, 1999).

Materials and methods

As source of yeast we used baker yeast from ROMPAK S.A. with 32.5% dry matter, and 46.54% protein content (N×5.7).

For the yeasts growing we used synthetics medium, containing various sources of nitrogen, phosphor, carbohydrates, minerals and vitamins.

The growth of the yeast was studied in the same conditions of time, temperature, pH and stirring.

As references it was considered the invertase activity of baker yeast (*Saccharomyces cerevisiae*).

One unit of invertase activity represents the number of inverted sugar micromoles released by hydrolytic action of one cm^3 crude enzyme preparation (or 1 g d.m.), during one minute in the following conditions: 20% sucrose as substrat, 0,02 M acetate buffer *p*H = 4.6, at 45°C.

Inverted sugar produced by hydrolysis at the moment of analysis was determinate by 3,5 DNS (dinitrosalycilic acid) reaction (Method of Analysis – AOAC

As essential medium (MB) for the yeast's growing we used industrial medium adapted to the laboratory conditions: $40cm^3$ wort, 0,08g $(NH_4)_2HPO_4$, 0,08g KH_2PO_4, 0,02g Mg $(NO_3)_2$ and 0,02g KNO_3. (Vasilescu,V. 1980).

The derivates mediums were:

- M1 – synthetic medium MB with 0.2 cm^3 lactoflavin, 0.2 cm^3 pyridoxine, 0.2 cm^3 thiamine, 0.2 g germs of wheat, 0.1 g wort extract and 0.08 g maize extract;
- M2 – synthetic medium MB with 0.2g germs of wheat, 0.1g wort extract and 0.08g maize extract;
- M3 – synthetic medium MB with 0.2g germs of wheat and 01g wort extract;
- M4 – synthetic medium MB with 0.2g germs of wheat and 0.08g maize extract;
- M5 – synthetic medium MB with 0.1g wort extract and 0.08g maize extract.

Starting from the methods of obtaining synchronous cultures proposed in the reference literature, four types of inoculum were used, such as:

- Inoculum 1^{st}: was commonly obtained from culture of 24 hour age on MMA;
- Inoculum 2^{nd}: was obtained by starving, that is the maintenance of a common inoculum into sterile distillated water for 24 hours;
- Inoculum 3^{rd}: was obtained by common inoculum thermostated at 28°C for 24 hours;
- Inoculum 4^{th}: was obtained by keeping the common inoculum at 0°C for 24 hours.

Results and discussion

With a view to stimulating the invertase biosynthesis in the yeast cells we studied the behaviour of *Saccharomyces cerevisiae,* which were suspended in five different media of culture, improves in some essential nutrients.

The cultivation was done submergently, on agitator (230 rot/min), at 28^0 C, for 24 hours.

A sucrose solution of 2% and a solution of NaOH (to regulate the *p*H) were added in these culture mediums.

The obtained biomasses were studied following the determination of humidity, dry substance and invertase activity.

The obtained results are expressed graphically in figure 1.

The obtained results were spectacular for all five synthetics media of cultures, but the most obvious was the valuable salt recorded on M1 and M3.

It could be said the best invertase activity was obtained through cultivation on super nutritive medium M1, when the increasing as compared the reference was by 13, 99 comparable with that on M3 medium (by 10).

Regarding from point of view of costs it could be said that is preferable to cultivate the yeasts cells on M3 medium, which is obvious more cheaply than M1 super nutritive medium.

So, it could be considered M3 as better culture medium, establish as a performing one, in order to continue our research.

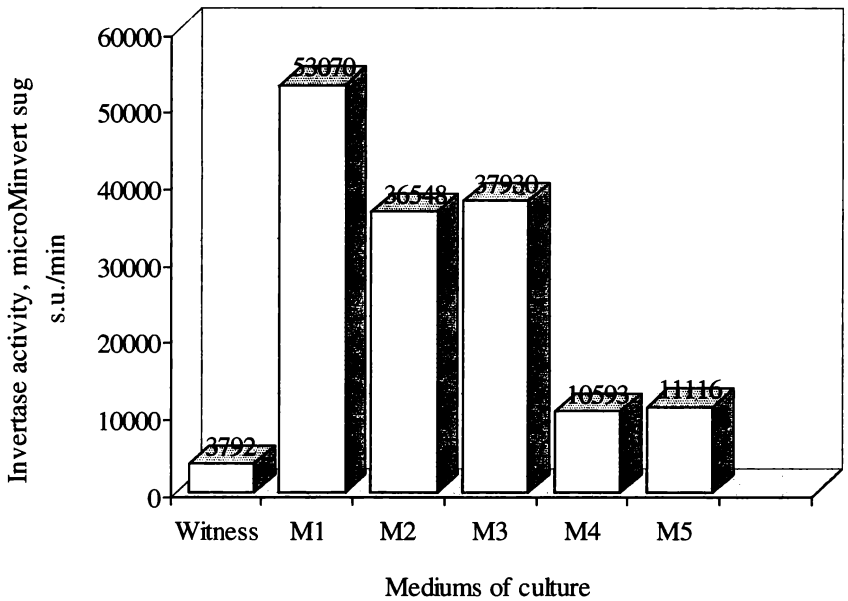

Fig. 1: The invertase activity obtained by submergently cultivation

Regarding to the types of inoculum it could be observed that the mechanism of these medium factors is unknown. It is considered that the modified factor synchronizes the synthesis of one or more components that generate cell division process.

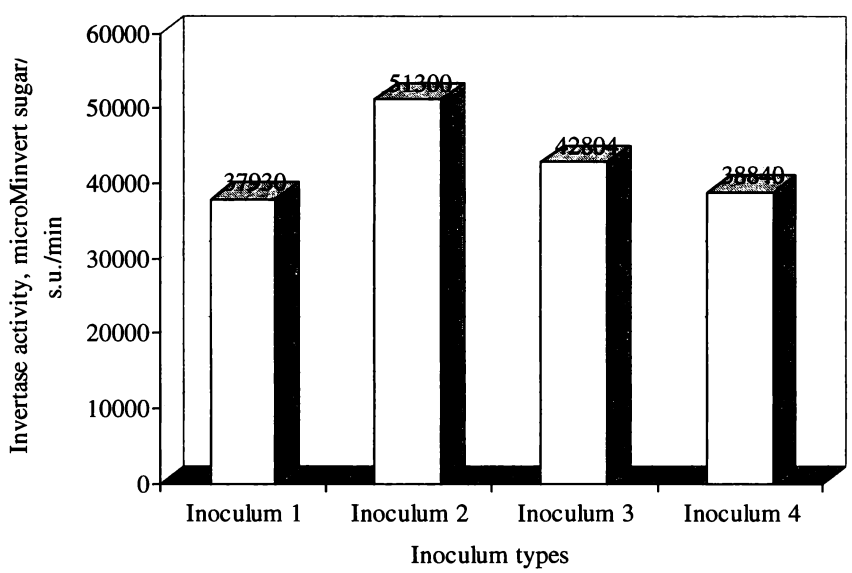

Fig. 2: Invertase activity variation of the strain *Saccharomyces cerevisiae* depending the inoculum type

All inoculum types were used by suspending in better nutritive medium M3. The cultivation was done submergently, on agitator (230 rot/min), at 28^0 C, for 24 hours.

A sucrose solution of 2% and a soluting of NaOH (to regulate the pH) were added in these culture mediums.

The biomasses recuperated by centrifugation at 4000 rot/min, for 20 minutes were studied from the point of view of invertase activity and cell growing capacity. The obtained results are expressed graphically in figure nr.2

The best results were obtained by using the starving inoculum through maintenance into sterile distilated water for 24 hours. In this case, the invertase potential increased 35.25% reaching almost the same value as in case of using common inoculum on the super nutritive medium M1.

Good results were obtained by using the inoculum thermostated at 28 ^{0}C for 24 hours where an invertase activity increase of 15.25% was made as compared to the activity registered by using inoculum 4, where the growth was of only 2%.

Conclusions

Analysing the obtained results from the study of outside factors action on invertase potential we can drop the following conclusions:

- Enzyme biosynthesis is greatly stimulated by using mixt exogenous additions, reaching increases of 111.5% as compared to witness medium
- The growth obtained in the case of super nutritive medium, but expensive, M1 can be equated by cultivating a cheaper medium, M3, of starving inoculum for 24 hours.

Bibliography:

Angehel. I. (1989). *Biologia şi Tehnologia drojdiilor*, vol. I, Editura Tehnică, Bucureşti.
Van Arx. J.A. (1980). in: *Biology and Activities of Yeasts.* Society for Applied Bacteriology Symposium, Series No. 9, Academic Press, London, New Zork, Toronto, Sydney şi San Francisco, 53 – 61.
Bahrim. G. (1999). *Microbiologie tehnică*, Editura Evrika, Brăila
Berry. D.R. (1982). *Biology of yeast,* The Institute of Biology's. Studies in Biology no, 140, Edward Arnold.
Burrows. S. (1970). *Baker's Yeast. The Yeast,* vol. III, Academic Press, London, New York.
Vasilescu. V. (1980). *Preparate enzimatice folosite în practică*, Editura Tehnică, Bucureşti,
Zamea. G., Mencicopschi, Gh. Brăgărea (1983). *Bioingineria preparatelor enzimatice.* Editura Tehnică. Bucureşti.