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# PRODUCTION OF YEAST PROTEIN BY *CANDIDA UTILIS* FROM PINEAPPLE JUICE
## II. BATCH CULTURE STUDY

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**Summary**

*The effect of aeration during batch cultivation of* ***Candida utilis*** *on yeast yield and its protein content was studied in a fermenter using the pineapple juice medium supplemented with $(NH_4)_2SO_4$ and $KH_2PO_4$ at 0.5% each. The total volume of the medium was 6 liters for each run. The agitation speed of the fermenter was kept at 1400 rpm. It was found that the yeast yield after 8 hours cultivation was highest (49.4%) at the aeration rate of 1.0 volume of air per volume of culture medium per minute (VVM). The protein contents of the yeast are the same for aeration rates of 0.5, 1.0 and 1.5 VVM. The reduction of chemical oxygen demand (COD) in the supernatant liquor was also determined during cultivation of the yeast. At the end of 8 hours cultivation with 1.0 VVM aeration rate, the COD reduction was in the range 70-80%.*

## Introduction

Using information obtained from the shake culture studies of *C. utilis*[1] the effects were investigated of aeration and agitation on the yield, protein content, sugar consumption, and reduction of chemical oxygen demand (COD) of the selected medium in a fermenter. Such information should be helpful when evaluating the usefulness of *C. utilis* as a means of utilization large quantity of pineapple juice for protein production.

## Materials and Methods

The yeast strain *(Candida utilis)*, the preparation of medium, and inoculum were described in the preceding paper[1]. Batch cultivation was carried out in a fermenter made of stainless steel with a plastic viewing panel, and agitation and aeration devices. The impeller on the agitator shaft was on open turbine type[2]. The diagram of the fermenter, designed after Finn[3], is shown in Fig. 1. The entire

Abbreviation: VVM, volume of air per volume of culture medium per minute; COD, chemical oxygen demand.

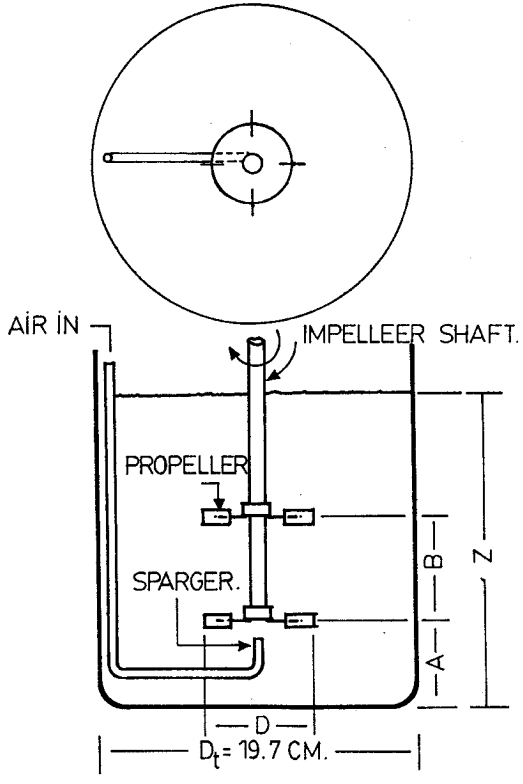


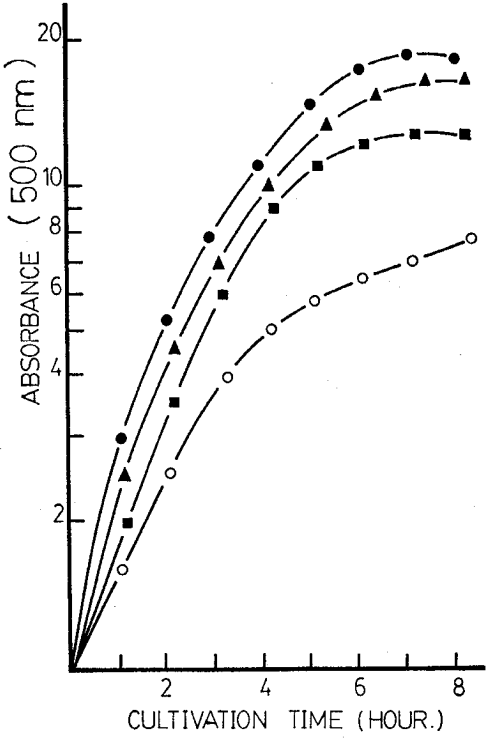

**Fig. 1.** Dimension of a stirred fermenter: Z/D = 10 D/D = 0.34; A/D = 0.8 to 1.0; B/D = 1.0 to 1.2

fermenter, accessories, and tubing were sterilized by 5% (v/v) phenol solution. The sterilized medium and inoculum were then added into the fermenter. The medium (medium 1) contained 2% sugar, 0.5% ammonium sulfate and 0.5% potassium dihydrogenphosphate. The total volume of the medium was 6 litres for each run. The culture was aerated with compressed air sterilized by filtration through a packed column (2.2 x 3.5 cm.) of sterile cotton filter. The air was introduced through this filter to the sparger located under the impeller. The sparger directed the air up into the impeller through 0.1 cm hole. The agitation was kept constant at the maximum speed of 1400 rpm. The aeration was controlled by a rotameter at the rates of 0.5, 1 and 1.5 volume of air/volume of culture medium/minute (VVM). Foaming during yeast cultivation was controlled, when required, by manual addition of sterile 10% (v/v) silicone solution. The pH during cultivation was maintained at 4.0 by manual addition of 6 N sodium hydroxide over 2 hours interval. Growth of *C. utilis* during cultivation in fermenter was measured every hour by absorbance reading at 500 nm. Two 25 ml of samples of the culture were withdrawn from the fermenter at every two hours for determination of dry weight of yeast cells. A quantity of contrifuged yeast cells was washed with distilled water, recontrifuged, dried in oven at 100°C, and kept for determination of protein content. The supernatant obtained from the determination of dry weight was collected for analysing sugar consumption and chemical oxygen demand (COD) in the culture medium. Fresh medium was added to compensate for the sample taken.

The concentration of sugar in the pineapple juice was determined by the method of Lane-Ennon[4], and expressed as percent invert sugar. The sugar content in the culture medium during cultivation was measured as percent invert sugar by the method of Stiles *et al.*[5]. The Kjeldahl nitrogen was analysed by the AOAC method[6] and yeast protein was calculated using a factor of 6.25[7]. The COD of the medium was determined in the supernatant liquor by the method of Porges *et al.*[8]. All determinations were made in duplicate. Percent yeast yield or conversion ratio was defined as dry weight of yeast cells obtained based on the actual sugar consumption in the medium[9].

## Results and Discussion

The amount of air supplied to a fermenter and the manner of its distribution are well known to be critical factors in the efficiency of yeast production by any process. In order to obtain high aeration rate on a large scale, aeration must be accompanied with agitation of the medium[10,11]. Agitation and aeration comprise a single effect because it is impossible to aerate all portion of a culture fluid without some degree of stirring[3]. This study was conducted in a stirred vessel fermenter, which gives a high rate of oxygen transfer, thus facilitating the rapid growth of yeast[12-14]. Since in highly stirred and aerated fermenter, microorganisms move regularly and fast enough to contact the substrate[15], high degree of aeration efficiency and agitation was found to permit very rapid utilization of sugar[10,16,17]. In this study the speed of agitation was kept constant at the maximum value of 1400 rpm.

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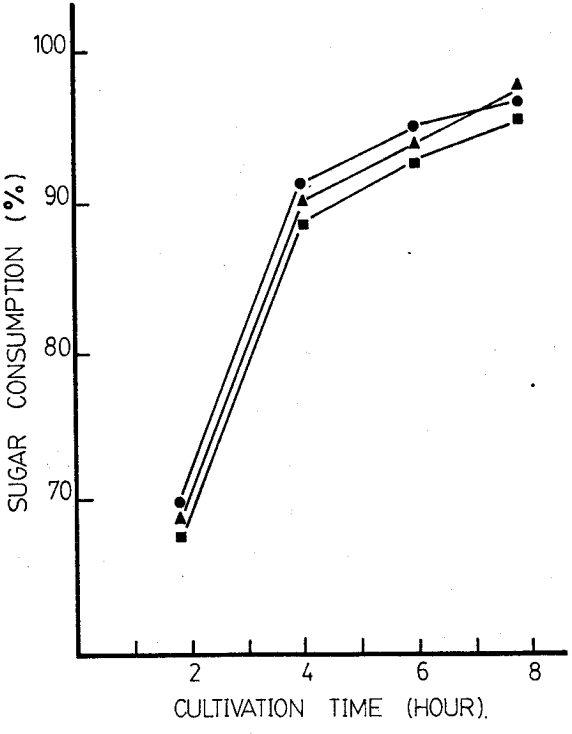


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**Fig. 2.** The growth curves of *C. utilis* in medium 1 at various aeration rates for batch culture experiments. The cultivation condition: pH = 4.0, temperature = 30°C, agitation speed = 1400 rpm. The medium composition: sugar = 2%, $(NH_4)_2SO_4$ = 0.5%, $KH_2PO_4$ = 0.5%. (■ – 0.5 VVM, ● – 1 VVM, ▲ – 1.5 VVM, O – shake flask study with medium 1 for comparison)

**Fig. 3.** The consumption of sugar in medium 1 during the growth of *C. utilis* at various aeration rates for batch culture experiments. The cultivation and condition medium composition– see Fig. 2. (■ – 0.5 VVM, ● – 1 VVM, ▲ – 1.5 VVM)

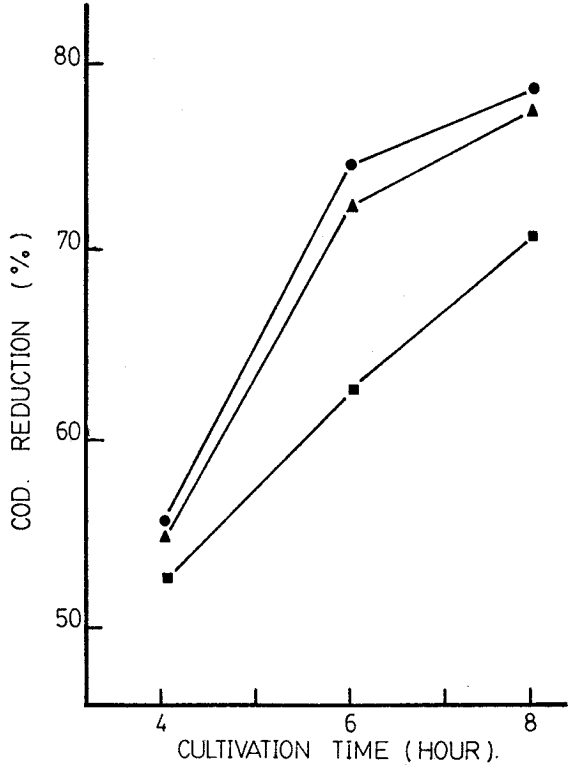


**Fig. 4.** The relationship between the reduction of chemical oxygen demand (COD) in the supernatant liqour and cultivation time during the growth of *C. utilis* in medium 1 at various aeration rates for batch culture experiments. The cultivation condition and medium composition—see Fig. 2. (■ —0.5 VVM, ● —1 VVM, ▲ —1.5 VVM)

TABLE I: COMPARISON OF YIELD AND PROTEIN CONTENT OF *C. UTILIS* GROWN IN MEDIUM 1 WITH BATCH CULTIVATION AT VARIOUS AERATION RATES

| | Aeration rates (VVM) | | |
|---|---|---|---|
| | 0.5 | 1.0 | 1.5 |
| Yeast dry weight, g/l | 8.70 | 9.50 | 8.96 |
| Protein content, % | 55.12 | 55.46 | 55.37 |
| Yield, % | 45.76 | 49.41 | 46.20 |

Only aeratoin rate was varied to determine the influence of aeration on yeast growth, yield, protein content, sugar consumption and reduction of COD as the function of cultivation time. The yeast was grown in medium 1 which was selected from the results of the previous experiments[1]. In Fig. 2, the growth curves of *C. utilis* medium 1 are shown at three aeration rates together with the yeast grown in the shake flask for comparison. The growth in the fermenter is superior than that in shake flask over the entire period of cultivation. It appeared that the aeration rate of 1 VVM gave the best growth. Apart from a proper composition of the fermentation medium, the amount of air required for efficient yeast growth is known to be critial[11]. Too much air can be accompanied by an increase of respiration and heat formation, and consequently low yeast yield. Too little air allows anaerobic fermentation condition to arise with an decrease of yeast yield in favor of alcohol production. Unfortunately, the efficiency of aeration is varied in different substrates and with differing types and sizes of equipment; hence it is rather difficult to make generalized specification of air requirement for yeast[2,3,11]. It has not yet been reported that yeast has an optimum aeration level. It must always be assumed that aeration limits the yield of yeast unless it can be shown that more effective aeration does not increase yields[10]. The change of aeration rates from 0.5 VVM to 1 VVM resulted in a better yield (see Fig. 2). But when the aeration was raised to 1.5 VVM, no improvement of the yield occured. So the best aeration obtained in the condition studied was 1 VVM judging from both growth curves and the yeast yields (see Table I). The result agrees with Rhodes and Fletcher[18], and Finn[3], who reported that air rate for yeast growth should not exceed 1 VVM for fermenter with an open impeller. The yeast yield obtained from the shake flask was 42.1% at the end of 64 hours, whereas the value of 49.1% was obtained when yeast was grown in the same medium but in fermenter at the end of only 8 hours. The important effect was caused by agitation and aeration. Harris *et al.*[12] reported that on the production of *C. utilis* on wood hydrolyzates, the yield was about 42%. *Torula* yeast grown on potato starch waste water gave cell yield of 45%[16] whereas the same organism grown on molasses gave the yield about 56%[19]. In addition to the necessity of the balance composition of the medium, the yield of *C. utilis* was a function of the concentration of the sugar present in the medium[9,20,21]. Cell yields of *C. utilis* grown on citrus press liquor varied from 70 to 26% depending on the initial concentration of

sugar. The high cell yield of 70% was obtained with 0.6% initial sugar concentration, and the low yield of 26% occurred with 3.8% sugar present initially[20]. The yield of *C. utilis* from citrus-waste press juice decreases from (44.3-48.0) to (37-37.7)% when the total sugar content of the juice was increased from 1 to 1.8%[21]. It was reported that use of the press juice containing not more than 1.0% total sugar seemed to be the most desirable because propagation was completed faster and the yields were higher. Not much change was obtained in the protein content of *C. utilis* grown in both shake flask and fermenter (about 55.38%, see Table I). The result agrees well with other studies[22, 23] which reported that aeration and agitation had negligible effects on the protein content of yeast. *Torulopsis utilis* grown on citrus-waste press juice yielded a product with 55.28% protein[21]. This organism when grown on molasses contained 55.9% protein[24] and 55% protein when grown on the protein waste water from potato[16]. In general, *C. utilis* grown on various raw materials such as sugar beet, sugar cane molasses, sulfite liquor, potatoes, corn cobs, paraffin, etc. gave protein content of 45 to 57% on dry weight basis[25, 26]. In this study, the quality of yeast protein was not determined. However, Reyes and Cassas[27] concluded that *C. utilis* propagated on waste of pineapple canning contained significant amount of essential amino acids. The values obtained compared favorably with those found in food yeast grown on sulfite waste liquors. It was stated, however, that the protein quality of yeast grown on carbohydrates, regardless of sources (molasses, cellulose, sulfite waste liquor, etc.) would be superior than any other cheap proteins, e.g., bacteria and algae[28].

All of the sugar consumption curves obtained in this study are biphasic, with a rapid phase followed by a show phase (Fig. 3). However, the sugar consumption was almost 100% for the three aeration rates studied at the end of 8 hours cultivation. The rate of sugar consumption in fermenter was exceedingly higher than that in the shake-flask experiments[1]. This was due to the inadequate amount of air supplied to the yeast grown in the shake flask. In general, the sugar consumption results in COD reduction. During cultivation of *C. utilis* at three aeration levels, the reduction of COD was achieved (Fig. 4). In this study, the highest COD reduction (78%) was obtained when *C. utilis* grown on medium 1 at aeration rate of 1 VVM. This corresponds to the condition where the highest yeast yield was obtained (see Table I). The COD reduction obtained in this study is slightly higher than in any previous reports. For example, *C. utilis* cultivated in tapioca starch waste water removed 73% of the soluble COD[29] whereas that grown on molasses spent wash gave 37-57% COD reduction[30]. Yeast production was intended to be used as partial solution to the waste disposal problem in many effluents of agricultural wastes, i.e., molasses, spent sulfite liquor and potato wastes[20, 31-33]. The operating conditions and some limitations of the designed fermenter used in this study did not permit the determination of optimum condition for yeast yield and COD reduction. Batch cultivation generally starts with a very slow rate of growth, known as induction period[16]. Continuous culture gives better control, shorter retention period and lower operating costs[29]. Nevertheless, the preliminary results obtained in the present work serves to demonstrate the possibility of propagation of *Candida utilis* on pineapple juice. With this technique dissolved materials such as sugar,

phosphate, nitrogen and many other substances in the medium can be converted into a useful yeast protein, which is attracting a growing interest as a contribution to the protein malnutrition. This process could lead to a worthwhile utilization of organic nutrients in other waste effuents, thus providing a better means for lowering their pollution potentials.

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## บทคัดย่อ

ได้ศึกษาผลของการให้อากาศต่อการเพาะ *Candida utilis* แบบแบทช์ (batch) ในเครื่องหมักโดยใช้น้ำสับปะรดที่เติม $(NH_4)_2SO_4$ และ $KH_2PO_4$ อย่างละ 0.5% ลงไปด้วย ปริมาตรรวมเท่ากับ 6 ลิตร ความเร็วของการกวนเท่ากับ 1400 รอบต่อนาที ได้พบว่าผลผลิตยีสต์ หลังจาก 8 ชั่วโมง จะสูงสุด (49.4%) เมื่อให้อากาศในอัตรา 1.0 ปริมาตรอากาศต่อปริมาตรน้ำเลี้ยงต่อนาที (VVM) ปริมาณโปรตีนเท่ากัน เมื่อให้อากาศในอัตรา 0.5, 1.0 หรือ 1.5 VVM ได้ตรวจสอบการลดของ chemical oxygen demand ในน้ำเลี้ยงส่วนบนด้วย และพบว่าหลังจาก 8 ชั่วโมง เมื่อให้อากาศในอัตรา 1.0 VVM จะมีการลดนี้ในช่าย 70-80%