THE EFFECT OF MOLASSES FOR LYSINE PRODUCTION BY *Arthrobacterium citreus NRRL 1258* AND *Corynebacterium glutamicum* ATCC 21475

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ABSTRACT

The essential amino acid L-lysine was produced in a batch fermentation by *Arthrobacterium citreus* NRRL 1258 and *Corynebacterium glutamicum* ATCC 21475. Molass ,a food by-product from a sugar mill, was chosen for this study. The main objective was to replace synthetic glucose with molasses in order to make use of this by-product and also to reduce total production costs of L-lysine. The results revealed that the growth of *Arthrobacterium citreus* NRRL 1258 and *Corynebacterium glutamicum* ATCC 21475 did not follow the simple Monod's kinetics. Growth inhibition by the product (L-lysine) occured during fermentation. Substrate (molasses) inhibition at higher concentrations (100, 120 g/l) ware exhibited against growth. The concentration of 40 g equivalent sugar/l molasses gave the highest lysine yield of 25.14 g/l for *Arthrobacterium citreus* NRRL 1258 and of 24.95 g/l for *Corynebacterium glutamicum* ATCC 21475. In addition, the statistical analysis showed that there was no significant difference of $P>0.05$ between molasses (40 g/l) and the synthetic glucose (80 g/l). This study clearly indicate that molasses (40 g/l) was a carbon suitable source in the lysine production process.

KEYWORDS: Molasses, *Arthrobacterium citreus, Corynebacterium glutamicu,* Lysine Production

1. INTRODUCTION

Lysine, an essential amino acid, is nutritionally important to men and animals and can be used as supplement food and food materials especially cereal products to improve protein quality [1]. It is the third most frequently produced amino acid. It is a large industrial scale, which is commercially manufactured. The largest amount (80%) of lysine is produced by fermentation and the remaining (20 %) is produced by chemical synthesis [2]. The culture media used for L-lysine production normally contain carbon and nitrogen sources. The usual inorganic nutrients are available from potasium phosphate, magnesium sulphate, manganese sulphate , zinc sulphate, ferrous sulphate and sodium chloride [3]. The suitable carbon sources are carbohydrates such as glucose, fructose, sucrose and starchhydrolysate, molasses and organic acids [2]. Millet sugar and defatted meals can be served as good carbon and nitrogen sources for high lysine yield production [4]. Oil seed meals, the by products of oil extraction, are potentially useful low-cost substrate-serving as nitrogen sources for the production of different substances [5]. Molasses are desirable raw material for L-lysine fermentation because of its availability and relatively low price. The application of acetate as a substrate for lysine synthesis could be more attractive in comparison with glucose during the lysine synthesis phase when the bacterial growth rate is below its maximum. Excess energy can be produced in cells on glucose [6]. Several strains of *Corynebacterium sp.* and *Brevibacterium sp.*, now collectively known as *Corynebacterium glutamicum* are used for industrial production. [7]. [8] used the carbohydrates sugarcane juice, molasses, banana, cassava and coconut water as sources of sugar/carbon for methionine production by microbiological methods. [9] was studed the effect of carbon source on microfiltration of

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Corynebacterium glutamicum. The result was investigated, changes in carbon source for the fermentation of sugar to lysine affected filtration performance. Relatively to glucose, the specific resistance of cells cultivated with sucrose was half as much at neutral pH, and almost the same below pH 4. These differences in specific resistance, as well as their pH dependencies, can reasonably be attributed to the higher hydrophobicity and lower surface charge of cells grown on sucrose. The same surface properties can not account for the much greater increase of cake resistance observed for cells grown in molasses medium. The present work is concerned with the optimization of cultural conditions for enhancing the production of L-lysine yield by strains of *Arthrobacterium citreus* NRRL 1258 and *Corynebacterium glutamicum* ATCC 21475.

2. MATERIALS AND METHODS

2.1 Culture cultivation

Ten percent of the inoculated seed medium (which was grown at 30°C for 18 hours in 50 ml in a 250 ml Erlenmeryer flask and put on a rotary shaker at 200 rpm) was used to inoculate a culture medium. Batch cultures were carried out for 90 hours under the following experimental conditions: 30°C and shaking 250 rpm speed. Samples were taken at different times every 6 hours. The pH, dry cell mass and fermentation broth were analyzed for the concentrations of the nitrogen, residual glucose and L-lysine.

2.2 Analytical methods

The L-lysine producers were *Arthrobacterium citreus* NRRL 1258 and *Corynebacterium glutamicum* ATCC 21475. Cell density was determined by dry weight after centrifugation at 5000 rpm for 20 min, washing twice with water and drying at 105 °C for 24 hours. Glucose concentrations were determined colorimetrically by using the Somojai method. and nitrogen concentrations were determined by the Kjeldahl method. L-lysine concentrations were determined by high-performance liquid chromatography (HPLC) with UV-VIS Detector. Separation was achieved using an C18 analytical column (150x4.6 mmI.D.). Sample injections of 20ÀµI The buffer gradient consisted of 0.2ÀM potas sium dihydrogenphosphate , pH 2.5 . the flow rate, 1 ml/min. L-lysine was quantified using a standard reference compound at various concentrations. The statistical analyses (SPSS-11) were based on Duncan's range tests.

2.3 Microorganism strains

The L-lysine producing strains of *Arthrobacterium citreus* NRRL 1258 and *Corynebacterium glutamicum* ATCC 21475 were selected as L-lysine producers in this study. It was obtained from the growth curve studied and produced L-lysine in lysine production medium. Both strains can produce L-lysine at the maximum yield when they were carried out for 72 hours.

2.4 Fermentation media

The seed medium contained per liter of distilled water of the followings: 20 g glucose, 5 g yeast extract , 2.5 g NaCl, 300 µg thiamine-HCl and 400 µg biotin. Its pH was adjusted to 7.2. For L-lysin e production, the molasses was used as a medium contained per liter of distilled water, $(NH_4)_2SO_4$ 75 g , K_2HPO_4 1.0 g, yeast extract 20 g , KH_2PO_4 1.0 g , $MgSO_4.7H_2O$ 0.4 g, $MnSO_4.4H_2O$ 10 mg, $FeSO_4$.7H2O 10 mg, thiamine-HCl 300 µg, biotin 400 µg. Its pH was adjusted to 7.4 with 1 M NaOH. The medium was sterilized by autoclaving for 15 min at 121 °C. Vitamins were sterilized separately by autoclaving and by membrane filtration (0.45µm). Molasses was diluted with distilled water. It was previously centrifuged and sterilized at 110 °C 15 min and used as fermentation substrate. The molasses was used at the amount of 40-120 g. The lysine medium was used as a control and had the same composition as a fermentation except that the molasses are substituted by glucose (80 g).

3. RESULTS AND DISCUSSION

3.1 The growth curves of *Arthrobacterium citreus* NRRL 1258 and Corynebacterium glutamicum ATCC 21475 in molasses medium

The results obtained reveal the time courses of pH, dry cell mass, L-lysine production, nitrogen and reducing sugar consumption in a medium. The biomass concentration increased and the reducing sugar concentration was declined continuously while the concentration of L-lysine increased towards 78 hours.

The reducing sugar consumption decreased continuously at 24.18 g/l for *Arthrobacterium citreus* NRRL 1258 and at 22.25 g/l for *Corynebacterium glutamicum* ATCC 21475. The reducing sugar consumption profile was substantially modified in conditions due to the increase in biomass. The maximum amount of L-lysine (23.59 and 22.49 g/l) was achieved when the initial pH of fermentation medium dropred at 6.17 and 6.14 of *Arthrobacterium citreus* NRRL 1258 and *Corynebacterium glutamicum* ATCC 21475 respectively. (Figures 1 and 2).

3.2 Effects of sugar concentrations

The effect of different sugar concentrations in molasses (40-120 g/l) on L-lysine productions by *Arthrobacterium citreus* NRRL 1258 and by *Corynebacterium glutamicum* ATCC 21475 was carried out at 72 hours .The maximum amounts of L-lysine produced at 25.14 g/l and 24.95 g/l were obtained in the medium containing 40 g/l eqivalent of sugar in molasses for *Arthrobacterium citreus* NRRL 1258 and for *Corynebacterium glutamicum* ATCC 21475, respectively (Tables 1 and 2).

The initial sugar concentration has been found to determine the amount of L-lysine produced. Reduction in L-lysine formation was observed when the sugar concentration of molasses was increased. In addition, the dry cell mass decreased when the sugar concentration increased. However, the amounts of nitrogen concentrations were different when the sugar concentrations were varied.

The sugar equivalent concentrations prepared from molasses directly affect the pH of fermentation media. The higher concentration of sugar equivalent lowers the pH of a fermentation medium, which limits the growth of lysine producing bacteria. As the results, the lysine yield is decreased, this can be observed by the decrease in the amount of dry cell mass.

The results showed that the final concentrations of dry cell mass, L-lysine, reducing sugar consumption and nitrogen and pH between media used of glucose and used of molasses.

Statistical analyses show that it was not significantly different ($P < 0.05$) in the amount of L-lysine production among different concentrations of molasses at 40, 60 and 80 g/l. both by *Arthrobacterium citreus* NRRL 1258 and *Corynebacterium glutamicum* ATCC 21475

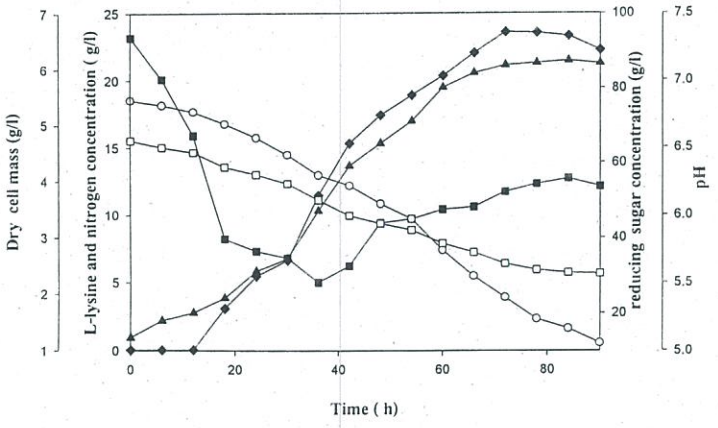

Figure 1. Kinetics of growth in 80 g/l molasses medium of *Arthrobacterium citreus* NRRL 1258(♦) L-lysine (g/l), (▲) dry cell mass (g/l), (○) sugar concentration (g/l) (□) nitrogen concentration (g/l), (■) pH

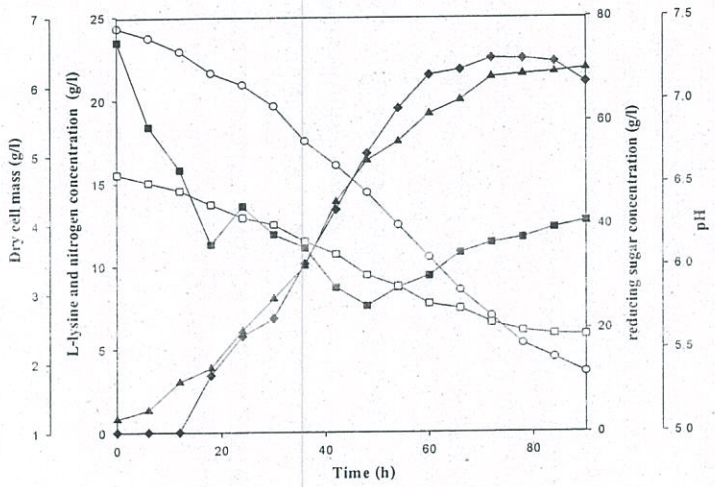

Figure 2. Kinetics of growth in 80 g/l molasses medium of *Corynebacterium glutamicum* ATCC 21475 (♦) L-lysine (g/l), (▲) dry cell mass (g/l), (○) sugar concentration (g/l) (□) nitrogen concentration (g/l), (■) pH

Table 1 L-lysine production, Dry cell mass, Reducing sugar, Nitrogen concentration and pH fermentation of *Arthrobacterium citreus* NRRL 1258 in molasses medium

Sugar concentration (g/l)	L-lysine production(g/l)	Dry cell mass (g/l)	Reducing sugar (g/l)	Nitrogen concentration (g/l)	pH
Control	26.09a	6.65a	19.08d	7.55c	6.53b
40	25.14a	6.53b	18.04e	7.71c	6.57a
60	24.48ab	6.28c	19.25d	7.16d	6.48c
80	22.84b	6.10d	21.34c	7.07d	6.22d
100	10.17c	4.40e	33.66b	9.37a	5.32e
120	8.04d	2.79f	43.46a	8.82b	5.25f

Table 2 L-lysine production, Dry cell mass, Reducing sugar, Nitrogen concentration and pH fermentation of *Corynebacterium glutamicum* ATCC 21475 in molasses medium

Sugar concentration (g/l)	L-lysine production (g/l)	Dry cell mass (g/l)	Reducing sugar (g/l)	Nitrogen concentration (g/l)	pH
Control	25.93a	6.53a	19.02d	7.06d	6.76a
40	24.95a	6.47ab	18.17e	7.93b	6.62b
60	23.53b	6.27ab	19.44d	7.52c	6.46c
80	22.30c	6.20b	20.52c	6.82e	6.15d
100	8.32d	4.60c	33.04b	8.87a	5.23e
120	6.46e	3.80d	44.44a	8.68a	5.06f

-Control = synthetic glucose medium, and 40,60,80,100,120, added sugar by molasses (g/l).
Means in the same column followed by a different letter (a,b,c,d,e) represent significant differences ($P < 0.05$) by DMRT.
-All values displayed are the average values in the 72 hr.of incubation

4. CONCLUSION

The study shows that *Arthrobacterium citreus* NRRL 1258 was able to produce L-lysine from molasses media .The L-lysine and dry cell mass were above 27.00 g/l and 6.60 g/l in molasses media with 40 g/l, sugar concentration respectively. The L-lysine and dry cell mass were above 24.73 g/l and 5.47 g/l in molasses media with 40 g/l sugar concentration for *Corynebacterium glutamicum* ATCC 21475. This result clearly indicates that molasses are potention sources of carbon and energy in L-lysine production.

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