ETHANOL PRODUCTION BY EFFECTIVE MICROORGANISM (M1) ISOLATED FROM SUGAR CANE FACTORY UNDER NON-STERILIZED CONDITION

SẢN XUẤT ETANOL BẰNG CHỦNG VI SINH VẬT HIỆU LỰC PHÂN LẬP TỪ NHÀ MÁY SẢN XUẤT ĐƯỜNG MÍA TRONG ĐIỀU KIỆN KHÔNG VÔ TRÙNG

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ABSTRACT

The effective microorganism (M_1) isolated from sugar cane factory in the Northern-East of Thailand was selected for ethanol production. Ethanol production from sugar cane molasses under non-sterilized condition was evaluated in batch fermentation. The highest yield of ethanol was achieved in molasses fermentation media containing 155 g/l total sugar, supplemented with 2.4 g/l $(NH_4)_2SO_4$, 1.2 g/l KH_2PO_4 and 0.1 g/l $MgSO_4$, in shaking flask (100 rpm) at room temperature after 48 h fermentation time. Comparison with sterilized condition, the same highest ethanol yield of 79 g/l indicated that no contamination occurred in the non-sterilized process.

Keywords: ethanol production, sugar cane, molasses, non-sterilized condition

TÓM TẮT

Một chủng vi sinh vật hiệu lực (M_1) phân lập từ nhà máy sản xuất đường mía ở Đông Bắc Thái lan đã được chọn để sản xuất etanol. Sản xuất etanol từ rỉ đường mía trong điều kiện không vô trùng được đánh giá bằng lên men từng mẻ. Hiệu suất etanol cao nhất đạt được trong khi lên men rỉ đường trong môi trường chứa 155 g/l đường tổng, bổ sung với 2,4 g/l $(NH_4)_2SO_4$, 1,2 g/l KH_2PO_4 và 0,1 g/l $MgSO_4$, lắc với tốc độ 100 vòng/phút ở nhiệt độ phòng sau 48 h lên men. So sánh với điều kiện vô trùng, cùng một hiệu suất etanol cao 79 g/l cho thấy quá trình không vô trùng không bị nhiễm tạp.

I. INTRODUCTION

Ethanol production as an alternative fuel energy resource has been a subject of great interest since the depletion of fossil fuel reserves and unstable of the petrol prices. Therefore, a strong need exists for efficient ethanol production with low cost in raw material and energy consumption. The development of a fermentation process using economical carbon sources is important for the biofuel ethanol production on a commercial scale [1, 2]. Many studies have been done that focus on production improvement and decreasing its costs [3-5]. Also, strains with efficient ethanol production have been studied [6-8]. Among several studies, ethanol production under non-sterilized condition has gained much attention of many researchers since it can save 30-40% energy consumption in sterilization process during ethanol production, which also makes process simper than ever before [9].

Molasses is an agro-industrial by-product containing fermentation sugars, being an optimal carbon source for the microorganism metabolism. Sugar cane molasses is an abundant agro-industrial material produced in tropical countries. In Thailand, approximately 2.5-3 Mt of molasses is produced, 60-70% of which is used for liquor and animal feed and around 1 Mt a year is reasonable to be used for ethanol fuel production [10]. Recently, the Thai government has set up a production target of 1.925 million liters a day of sugar-based ethanol [11].

By combining the energy economic of the non-sterilized process, it is possible to produce ethanol with lower cost and higher efficiency by effective microorganism (M_1) isolated from sugar cane factory. In this study, ethanol fermentation under non-sterilized condition by isolated M1 was conducted in sugar cane molasses medium.

II. MATERIAL AND METHODS

Microorganism and growth media

The strain used was M_1 which isolated from sugar cane factory in the Northern-East of

Thailand**.** The strain was maintained in YM agar plates containing (per liter): 10g glucose, 3g yeast extract, 3g malt extract, 5g peptone (Merck). The inoculum culture was prepared by transferring one single colony of effective microorganism to 50-mL Erlenmeyer flasks containing 15 ml of YM broth. The culture was incubated at 30°C with shaking rate of 150 rpm for 24 h. Then, 10% inoculum culture was transferred to growth medium consisted of 25% non sterilized sugar cane molasses supplemented with (gL^{-1}): $(NH_4)_2SO_4$ (2.4), KH_2PO_4 (1.2), and $MgSO_4.7H_2O$ (0.1). Non-sterilized sugar cane molasses media was prepared with distilled water. The strain was incubated aerobically with the same shaking rate of 150 rpm at 30°C for 24 h prior to use for the experiments.

Shaking flask experiments under non-sterilized conditions

In the first experiment, the influence of initial pH and the concentration of sugar cane molasses on the ethanol fermentation were investigated. Experimental studies have been done in shaking flasks under non-sterilized conditions at room temperature in fermentation medium containing 25 and 30% non sterilized sugar cane molasses containing (gL^{-1}): $(NH_4)_2SO_4$ (2.4), KH_2PO_4 (1.2), and $MgSO_4.7H_2O$ (0.1). The fermentation medium, each was inoculated with 10% (v/v) inoculum concentration. The initial pH was varied in the range of 4, 4.25, 4.5, 4.75 compared with non pH adjust (5.1). The shaking rate was 100rpm. Ethanol fermentations were conducted in 250ml Erlenmeyer flasks, each containing 100 ml fermentation medium, for 120h. The effect of shaking rate (50, 100rpm) and static condition, also the concentration of sugar cane molasses (20, 25, 30%) in fermentation medium were investigated in the second experiment under non-sterilized conditions. Ethanol fermentations were conducted in 250ml Erlenmeyer flasks, each containing 100ml fermentation medium without adjusted pH (5.1) at room temperature, for 72h.

Ethanol fermentation under non-sterilized and sterilized conditions

To study the characteristics of the non-sterilized process, fermentation media was prepared with distilled water in the container without autoclave sterilization. The autoclave process was accomplished with all implements, such as flask and media, at 110°C for 10min. Seed media were autoclaved and sterilized in both processes to prevent contamination in the seed culture. Ethanol fermentations were conducted in 500ml Erlenmeyer flasks, each containing 300ml fermentation medium without adjusted pH (5.1) at room temperature, for 72h. Every experiment was conducted in duplicate.

Analytical methods

The total sugar was estimated using Phenol Sulfuric acid method according to Dubois *et, al.* (1956) [12]. Ethanol analysis was conducted by gas chromatography (GC) Shimadzu, using gas column packed (PEG - 20M), column temperature 80°C, injection temperature 120°C and detector temperature 150°C.

III. RESULTS

To explore the potential of ethanol fermentation from sugar cane molasses under non-sterilized process by isolated M1, two factors of concentration in sugar cane molasses (25, 30%) and five main factors of initial pH (4, 4.25, 4.5, 4.75, without adjusted pH) of fermentation media, were investigated. Isolated M1 was cultured in non-sterilized media at different concentrations of sugar cane molasses and various initial pH which were adjusted by H2SO4. The initial total sugar (g/l) and ethanol production were measured (Table 1) during the fermentation period. At pH 5.1 (without adjusted pH) the highest ethanol yield of 79.87g/l was gained within 72h fermentation time in the concentration of 30% sugar cane molasses media containing of initial 155.23g/l total sugar.

The effect of shaking rate of 50, 100 rpm was compared with static condition in ethanol fermentation in non-sterilized medium without adjust pH (pH 5.1). The sugar consumption and ethanol fermentation is shown in Fig.1-2. The highest ethanol yield of 80.10 g/l was gained within 36h fermentation time in the concentration of 30% sugar cane molasses media containing of initial 152.22 g/l total sugar with a shaking rate of 100 rpm.

Comparison non-sterilized and autoclaved process, as shown in Fig. 3, the initial total sugar concentration of the media with the autoclaved process was 152.65 g/l similar to that of 155.35 g/l without autoclaving, which meant rarely of total sugar loss in autoclaved process due to sterilization temperature, was not too high. Ethanol production with non-sterilized and autoclaved processes was compared (Fig. 4). The initial pH of two processes was 5.1 without adjusted the fermentation medium. The fermentation was conducted in flask with the shaking rate of 100 rpm at room temperature. It was noted that 79.00 g/l ethanol yield was obtained at a total sugar concentration of 155.35 g/l within 48h in the non-sterilized process. For the autoclaved process, 78.79 g/l ethanol yield was achieved within the same time.

Table 1. Effect of concentration of sugar cane molasses, initial pH on the ethanol fermentation by Isolated M1

Treat	Experiments	Initial total sugar (g/l)	Max. yield of ethanol (g/l)	Fermentation time (h)
1	25% sugar cane molasses medium, pH 4.0	121.84	67.84	72
2	25% sugar cane molasses medium, pH 4.25	120.56	65.08	72
3	25% sugar cane molasses medium, pH 4.50	122.2	64.91	48
4	25% sugar cane molasses medium, pH 4.75	119.78	68.61	48
5	25% sugar cane molasses medium, without adjust pH (pH 5.10)	119.64	64.43	48
6	30% sugar cane molasses medium, pH 4.0	151.48	73.13	120
7	30% sugar cane molasses medium, pH 4.25	152.79	75.1	96
8	30% sugar cane molasses medium, pH 4.50	154.45	71.01	96
9	30% sugar cane molasses medium, pH 4.75	155.39	71.02	96
10	30% sugar cane molasses medium, without adjust pH (pH 5.10)	155.23	79.87	72

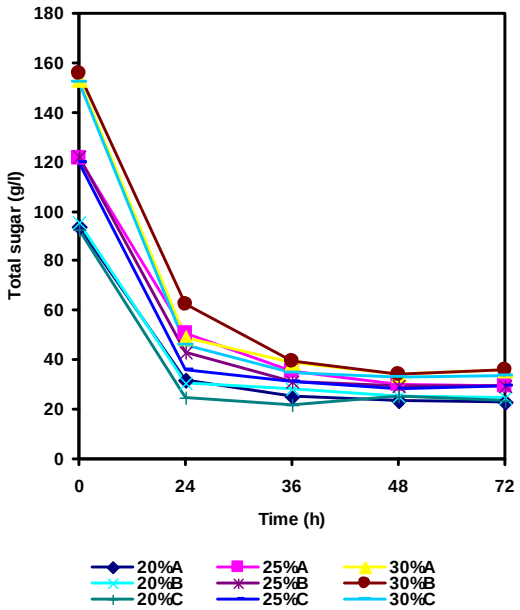

Fig.1 Sugar consumption with non-sterilized fermentation media in different concentration of sugar cane molasses, initial pH 5.1, at room temperature; A: static condition, B: shaking at 50 rpm, C: 100 rpm.

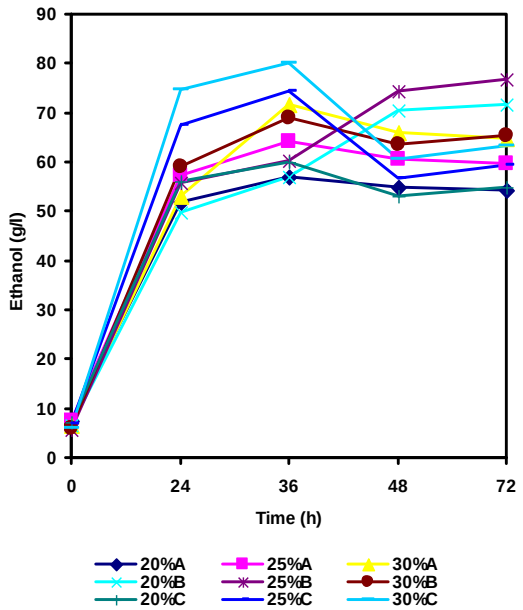

Fig.2 Ethanol fermentation with non-sterilized fermentation media in different concentration of sugar cane molasses, initial pH 5.1, at room temperature; A: static condition, B: shaking at 50 rpm, C: 100 rpm.

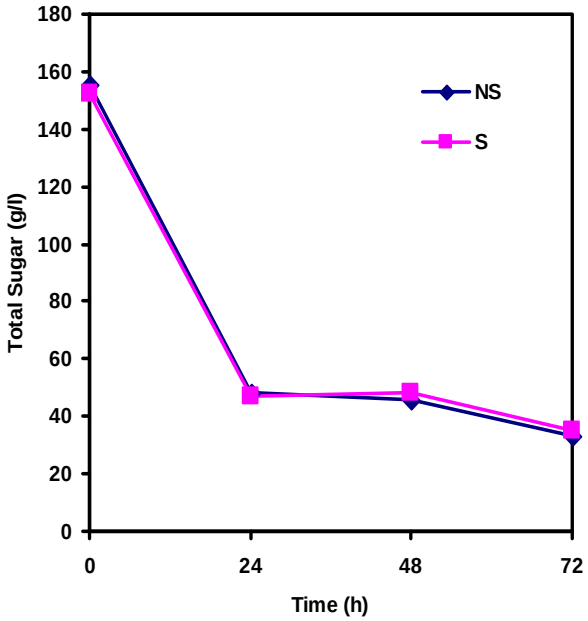

Fig.3 Sugar consumption with non-sterilized and autoclaved fermentation media in concentration of 30% sugar cane molasses, initial pH 5.1, 100 rpm, at room temperature; NS: non-sterilized, S: autoclaved medium.

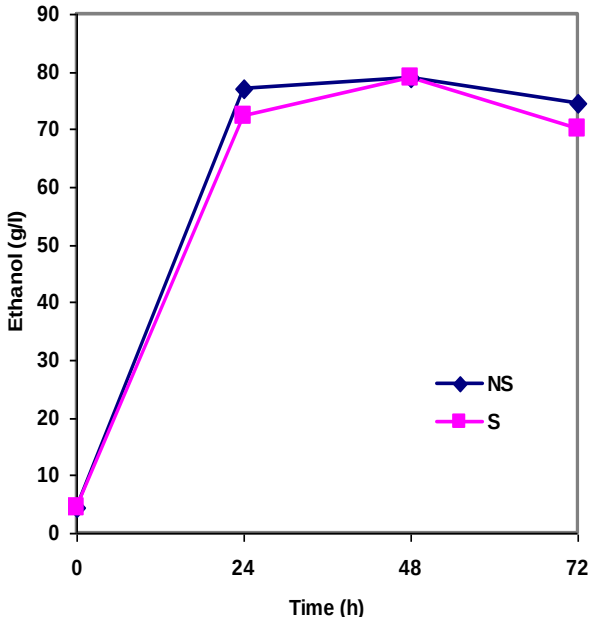

Fig.4 Ethanol fermentation with non-sterilized and autoclaved fermentation media in concentration of 30% sugar cane molasses, initial pH 5.1, 100 rpm, at room temperature; NS: non-sterilized, S: autoclaved medium.

IV. DISCUSSION AND CONCLUSIONS

High ethanol productivity and low energy consumption have been two important aspects of most ethanol fermentation researches. Ethanol production under non-sterilized condition will meet the demands of industrialization. The molasses was an industrial sucrose containing substrates that has been reported to contain substantial salt content which microorganisms can use for their metabolism [13, 14]. In the non-sterilized processes, there were no problems of wasting raw material. The initial sugar concentration was 155.35 g/l. After 48 h fermentation by isolated M1 in non- sterilized molasses media without adjusted pH (pH 5.1), incubated at room temperature with a shaking rate of 100 rpm, 79.0g/l ethanol was gained the same as the highest ethanol yield in autoclaved process.

Although low pH can control the potential contamination in the non-sterilized process, the highest ethanol yield in non-sterilized medium without adjust pH (pH 5.1) by isolated M1 was achieved within the same time of autoclaved process. However, microbes in the raw material are not killed. It is therefore necessary to take effective measures to prevent the ethanol fermentation from contamination. These are as follows: using excellent strains with acid tolerance and rapid cell growth; adjust pH of broth to no more than 4.5 to control the growth of the strains [15] or keeping the sanitation of the work environment**.** By employing these methods with effective microorganism, an efficient and less energy consuming ethanol production is possible in the near future.

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