Korean J. Chem. Eng., **29**(9), 1224-1231 (2012)
DOI: 10.1007/s11814-011-0293-0

Evaluation of sediments of the waste from beer fermentation broth for bioethanol production

Jung Hwan Ha*, Min-Kyung Gang*, Taous Khan*,, and Joong Kon Park*,†**

*Department of Chemical Engineering, Kyungpook National University, Daegu 702-701, Korea
**Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, Pakistan
(*Received 2 November 2011 • accepted 1 December 2011*)

Abstract−As our previous studies showed, the waste from beer fermentation broth (WBFB) is a potential resource for bio-ethanol production. The original WBFB was superior to the supernatant in this regard. The current study investigates the potential of the WBFB sediment alone for bio-ethanol production after it has been diluted with distilled water or a chemically-defined medium. The effect of stock time on WBFB sediments for ethanol production was also studied. The fermentations were carried out using 50 ml vials placed in a bioreactor in static conditions. There was relatively little increase in ethanol production with fermentation time (up to 2 h) and stock time (up to 7 days) using 20% (v/v) sediment in distilled water which did not contain any nutrients or enzymes. A 2.09% increase in ethanol production was recorded after 2 h fermentation with 20% (v/v) WBFB sediments (1 day old) in a chemically-defined medium. The increase was 3.25% for WBFB sediments with a stock time of three days in a chemically-defined medium. The results also showed some residual activity of starch hydrolyzing enzymes in the sediments, especially at 60 °C. The overall results of this study revealed that the sediments alone were capable of bio-ethanol production even though they were five-fold diluted with distilled water or the chemically-defined medium.

Key words: Bio-ethanol, Waste from Beer Fermentation Broth, Sediments, Fermentation, Saccharification

INTRODUCTION

There has been a tremendous interest in the development of alternate and renewable sources of energy in order to conserve the rapidly depleting reserves of crude oil and fossil fuels [1-3]. The increase in world population and urbanization has greatly increased the energy demand. Currently, the major source of energy in most countries is derived from fossil fuel, which is not a renewable source of energy, and its reserves are rapidly being depleted. Moreover, various environmental issues associated with the use of fossil fuels [3,4] have necessitated an urgent search for alternative energy sources to fulfill the current and future demand.

Bio-ethanol is rapidly becoming established as an alternative renewable energy source that can also overcome the environmental problems associated with the use of fossil fuels [5]. Although there are many other alternative fuels, such as methanol, methane, natural gas, propane, and hydrogen, the remarkable inherent characteristics of ethanol, including its high latent heat of vaporization, high octane number and rating, and lower emission of toxic compounds on combustion, have made it the best alternative fuel [2]. Bio-ethanol has various advantages over fossil and other fuels, yet its high cost of production is still a major issue to be addressed [6]. This issue can be solved by finding cheap raw materials with high potential for the production of bio-ethanol. To search for such cheap sources, various materials possessing sufficient carbohydrate contents have been tested for bio-ethanol production [5]. These materials include, but are not limited to, rice hulls [7], sugar cane leaves [8], bagasse [9], cassava stem [10], food waste [4], micro-algae [11], sorghum [12], straw [13], industrial waste [6], industrial, urban, and agriculture residues, and corn [14,15]. These sources contain various carbohydrates which are converted to simple sugars and glucose through saccharification, which requires various enzymes. These simple sugars are further converted to bio-ethanol by yeast and bacteria through fermentation [7]. However, most of these materials contain solid residue in the form of lignin and other nonusable raw materials, which limits their potential as efficient sources for bio-ethanol production [6].

In our previous studies [5], the waste from beer fermentation broth (WBFB) was evaluated for bio-ethanol production. These studies showed that WBFB is a potentially interesting resource for bio-ethanol production. The ethanol was produced from WBFB without any addition of enzymes, cells or nutrients. The WBFB contains about 25% (wet weight) of solid residue, mainly yeast cells, while the remaining 75% is liquid [5]. The analysis of the supernatant from WBFB showed that it contains high amounts of carbon, nitrogen [16] and other substances including enzymes and yeast cells [5], due to which it has been employed for the production of various high-tech products including bacterial cellulose [17,18] and water soluble oligosaccharides [16,19]. In previous studies [5], the original WBFB (a liquid with suspended/sediment particles) was found to be superior to the supernatant in terms of bio-ethanol production over a shorter period of time. These results clearly indicate the role of the solid residue in bio-ethanol production. The enzymes present in the supernatant of WBFB are actually derived from malted barley during the beer fermentation process [20,21] and are suitable for the saccharification process [5]. The WBFB is free from fungal strains producing saccharification enzymes [5,22], although sediments from the wastes of some traditional Korean liquor fermentation broths

†To whom correspondence should be addressed.
E-mail: parkjk@knu.ac.kr

contain fungi capable of producing such enzymes. Bio-ethanol was produced from WBFB by using a simultaneous saccharification and fermentation process (SSF) [5]. SSF is a common process for the production of bio-ethanol because it achieves higher yields by eliminating end-product inhibition and the need for separate reactors for saccharification and fermentation [8]. The process requires saccharification enzymes to convert polysaccharides into glucose and yeast cells that simultaneously transform the glucose to ethanol. The entire process occurs in the same reactor [23].

The current study has been designed to investigate the potential of solid residue (sediment) from the WBFB alone for bio-ethanol production. For this purpose, the sediment obtained from WBFB was subjected to bio-ethanol production after diluting with distilled water or a chemically-defined medium. The effect of the stock time on the WBFB used for obtaining sediment was also studied. The presence of saccharification activity in the WBFB sediments was also evaluated.

EXPERIMENTAL

1. WBFB and its Processing

The WBFB was obtained from the New Youngnam Hotel beer industry, Daegu, Korea and was stocked as reported previously [5]. The original WBFB was kept in closed cap containers at 20 °C and used to obtain sediments for the bio-ethanol production after various time periods (stock time). The container was shaken well in order to mix the contents uniformly before taking broth. The WBFB sediments were separated from the supernatant of by centrifugation for 20 min at 3,500 rpm using 50 ml centrifuge tubes.

2. Fermentations

Fermentations were carried out in 50 ml vials, each of which has a 50 ml working volume, under static conditions. The vials were placed into a beaker containing water which was itself placed into a double jacketed bioreactor with circulating water from a temperature-controlling water bath. The temperature of the bioreactor was allowed to increase from room temperature to 70 °C during fermentation. The profiles for the increase of temperature of the water bath from which the water circulated and the water in the beaker in which the vials were placed during fermentation are shown in Fig. 1. Samples were collected at different intervals and analyzed for bio-ethanol and other parameters. For this purpose, one vial was collected after 0, 30, 60, and 120 min of fermentation.

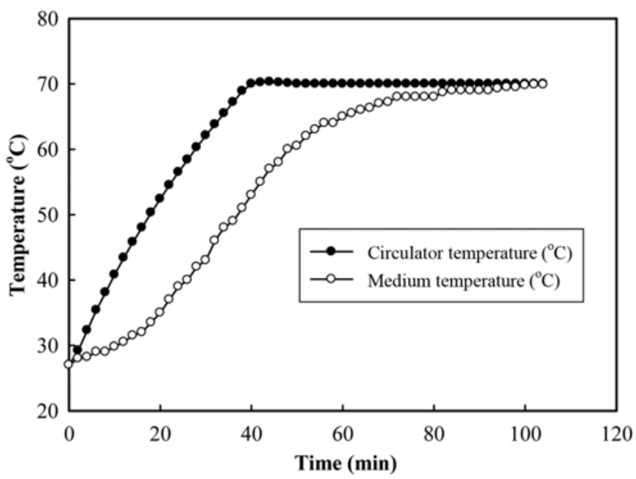


Fig. 1. Temperature profile during fermentation in static cultivation for the production of bio-ethanol using WBFB sediments in distilled water or chemically-defined medium.

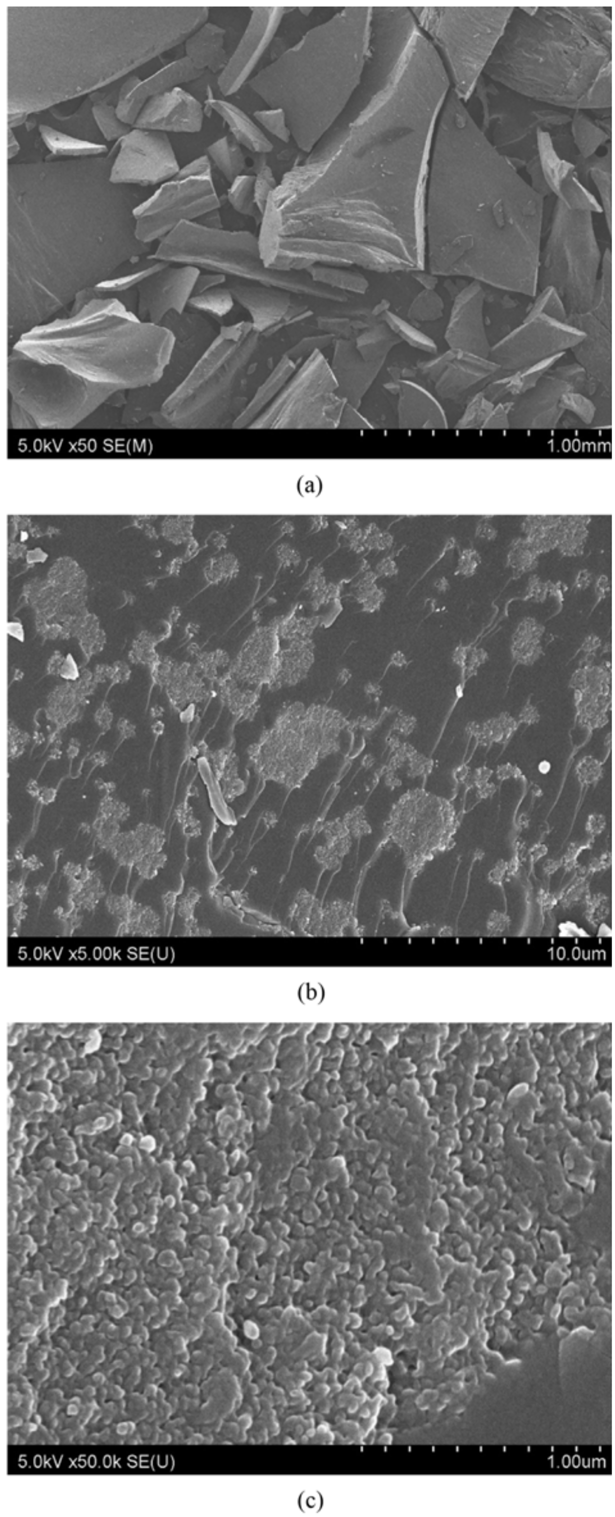


Fig. 2. FE-SEM micrographs of the freeze-dried sediments obtained from the WBFB by centrifugation at 3,500 rpm for 20 min at (a) 50×, (b) 5,000×, and (c) 50,000× resolution.

3. Saccharification Activity in Sediments Obtained from WBFB

To measure the residual activity of saccharification enzymes, sediments were obtained from the original WBFB by centrifugation at 3,500 rpm for 20 min. A 20% (v/v) mixture of the sediment was prepared in distilled water. Then 5% of soluble starch (Sigma-Aldrich, USA) was dissolved in this mixture and incubated at 150 rpm for two days at 30, 45 and 60 °C. The amount of glucose hydrolyzed from soluble starch during incubation was determined by a glucose analyzer (Refractometer ATGO® Japan) and a glucose kit (GAHK20 Sigma-Aldrich, USA).

4. Analytical Procedures

The concentration of ethanol was determined with a UV-visible spectrophotometer (model T60 U, Sunil Eyela Co., Ltd., made in China) using kit methods as reported previously [5]. For ethanol determination, the samples were first treated with a saccharide removal kit (Catalog #: DSRK-500, Saccharide Removal Kit, BioAssay Systems, Hayward, CA, U.S.A.) followed by an ethanol assay kit (Catalog #: DIET-500, QuantiChrome™ Ethanol Assay Kit from BioAssay Systems, Hayward, CA, U.S.A.) and finally the concentration was measured spectrophotometrically at 580 nm [24]. The total glucose concentration was determined by using a glucose analyzer (ATAGO® POCKET REFRACTOMETER, Pocket PAL-15S, Japan). The pH of the samples was determined using a pH meter (EUTECH INSTRUMENTS pH 510, Malaysia).

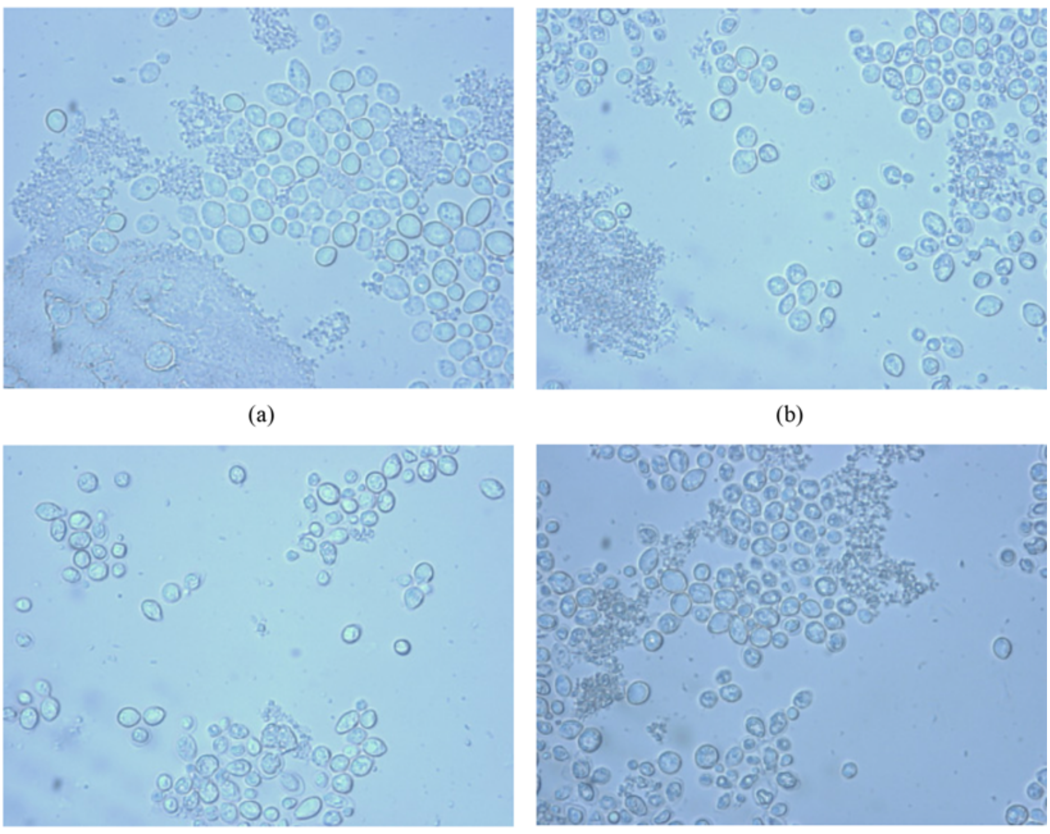
5. Microscopic Analysis of the Sediments and Culture Broths

The freeze-dried samples of the sediments obtained from the original WBFB by centrifugation at 3,500 rpm for 20 min were subjected to FE-SEM analysis in order to determine their morphology and possibly their physical composition. Micrographs of the platinum-coated sediments were taken with a field-emission scanning electron microscope (S-4300; Hitachi Co., Japan). Similarly, microscopic pictures were taken with a fluorescence microscope (JENAMED Germany) of the culture broths during static cultivation using 20% (v/v) sediment in distilled water and 20% (v/v) sediment in a chemically-defined medium at 0, 30, 60, and 120 min of cultivation.

RESULTS AND DISCUSSION

1. Microscopic Analysis of Sediments and Culture Broths During Fermentation

The freeze-dried samples of the sediments were subjected to FE-SEM analysis in order to investigate their morphological characteristics. Fig. 2 shows the SEM micrographs of the sediment obtained from WBFB by centrifugation for 20 min at 3,500 rpm followed by freeze-drying. These micrographs revealed the morphology of the sediments at various resolutions. Our previous studies [5] found that various yeast and bacterial strains are present in the WBFB, including *Saccharomyces cerevisiae*, *Candida krusei*, *Pediococcus dextrinicus* and *Brevibacterium verniforme*. It has also been reported that wine sediments are constituted of yeast cells (alive and dead) together with tartrates, proteins, polysaccharides, and other materials which precipitate in the tank as the alcohol concentration increases



(a) (b) (c) (d)

Fig. 3. Microscopic pictures taken during static cultivation at 67 °C of the mixtures of 20% (v/v) WBFB (0 day) sediment in distilled water at 500× resolution at (a) 0 min, (b) 30 min, (c) 60 min, and (d) 120 min.

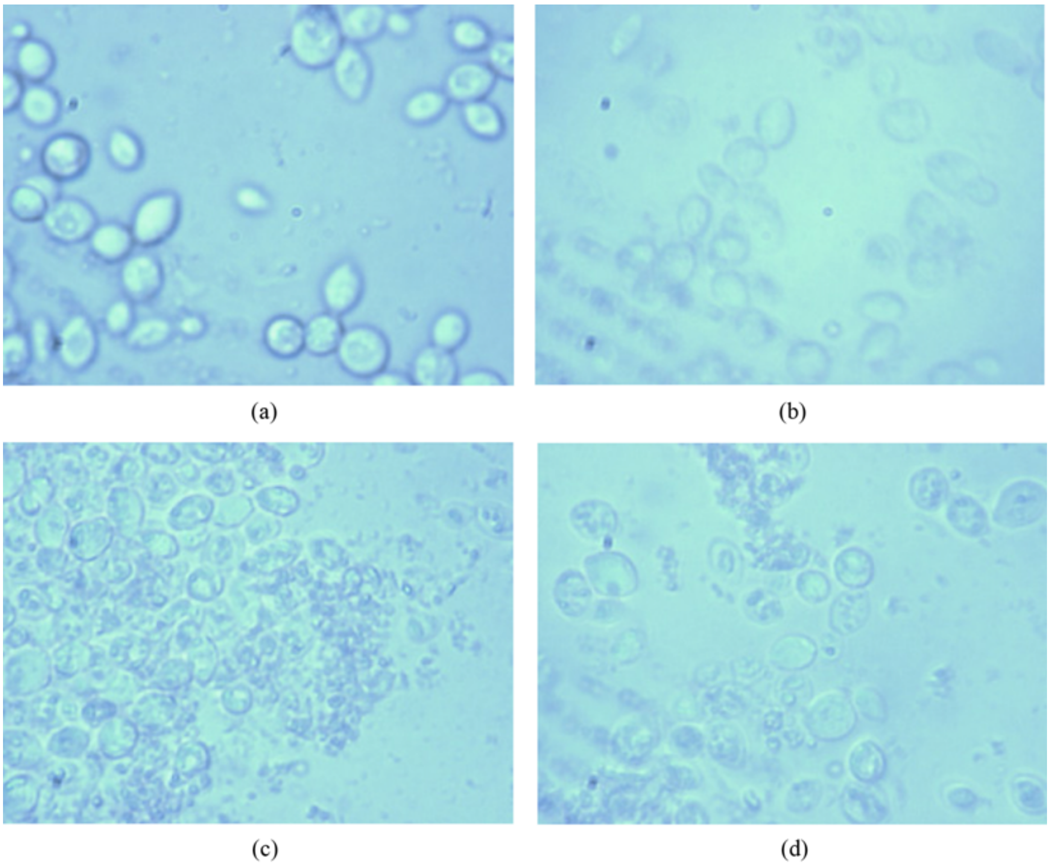


Fig. 4. Microscopic pictures of culture broth during static cultivation at 67 °C of the mixtures of 20% (v/v) WBFB (1 day) sediment in a chemically-defined medium (Glucose 100 g/L, Yeast extract 8.5 g/L, NH_4Cl 1.3 g/L, $CaCl_2$ 0.06 g/L, $MgSO_4$-$7H_2O$ 0.12 g/L) at 1,000× resolution at (a) 0 min, (b) 30 min, (c) 60 min, and (d) 120 min.

[25]. The SEM micrographs of sediment at lower magnification (×50) revealed flake-type fragments of various sizes. Similarly, the higher magnifications (×5.0 K and ×50.0 K) showed that these fragments have porous surfaces and seemed to be composed of small agglomerates. However, no yeast or bacterial cells, which are present in WBFB sediments [5,25], are clearly visible in these micrographs.

Similarly, our previous studies also indicated that yeast cells were predominant initially but decreased with increasing stock time so that other cells became dominant [5]. As shown in Fig. 3, the number of yeast cells in samples of culture broth composed of 20% sediment in distilled water remained almost constant throughout two hours of fermentation. As no or very few colony forming units were observed in the culture broth during fermentation (data not shown), it is expected that these are dead or deactivated yeast cells. Only a small amount of saccharification enzymes may be present in the broth because it is composed of five-fold diluted sediments in distilled water. The concentration of enzymes present in sediments similarly decreased during the dilution. This means that there is little external action upon these yeast cells except for heat treatment, and thus they remain undamaged even after 2 h of cultivation. Although the exact temperature at which the yeast cells lose their physical integrity is not known, *S. cerevisiae*, the dominant species in the WBFB [5], can grow at high temperatures within the *Saccharomyces* genus. Recently, it was found that *S. cerevisiae* grows best at 32.3 °C while 45.4 °C is its maximum growth temperature [26]. Fig. 3 shows that the fermentation temperature (67 °C) has very little effect on the physical integrity of the dead yeast cells when 20% WBFB sediments in distilled water are used as the culture broth. Fig. 4 shows

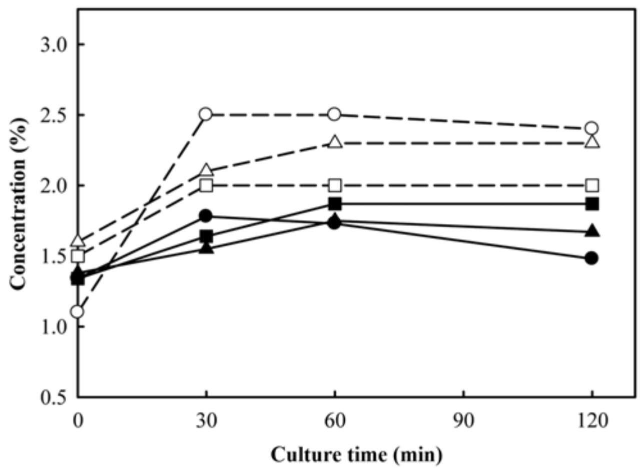


Fig. 5. Production of ethanol and glucose in static conditions using 20% WBFB sediments in distilled water contained in a 50 mL vial at 67 °C for 2 h. The solid lines (—) represent the ethanol concentration while the dashed lines (---) glucose concentration for WBFB at zero stock time (●, ○), 5 days stock time (■, □), and 7 days stock time (▲, △).

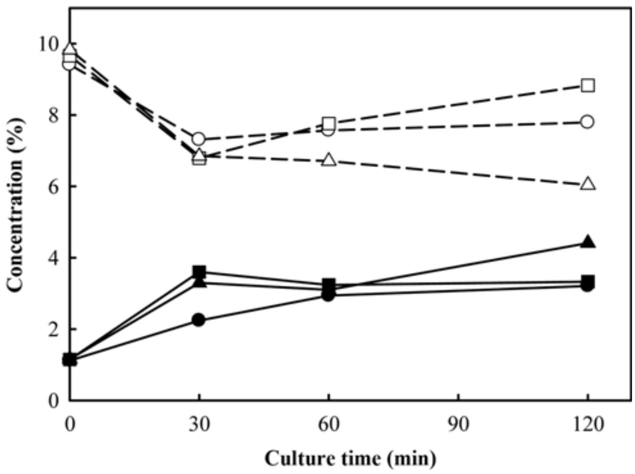


Fig. 6. Ethanol production in static condition using 20% (w/v) of sediment in a chemically-defined ethanol production medium (glucose 100 g/L, yeast extract 8.5 g/L, NH_4Cl 1.3 g/L, $CaCl_2$ 0.06 g/L, $MgSO_4$-$7H_2O$ 0.12 g/L) contained in a 50 mL vial at 67 °C for 2 h. The solid lines (—) represent the ethanol concentration while the dashed lines (---) glucose concentration for WBFB at 1 day stock time (●, ○), 2 days stock time (■, □), and 3 days stock time (▲, △).

microscopic pictures of the yeast cells in samples of a culture broth composed of 20% sediment in an ethanol producing chemically-defined medium. The wear and tear on the yeast cells at 30, 60 and 120 min (Fig. 4(b)-(d)) can be clearly seen in these pictures as compared to the cells at the beginning of the fermentation (Fig. 4(a)), although their number may remain constant. The damage to the cells may be due to the higher ethanol concentration [27] in the culture broth (Fig. 6) compared to the culture broth composed of 20% sediment in distilled water (Fig. 5). Ethanol changes the degree of polarity of the cell membrane and the cytoplasm and thus causes disruption of growth at higher concentrations [5,28,29]. This disruption may be in the form of increased membrane fluidity [30]. The effect of disruption may be further accelerated due to the higher temperature of fermentation (67 °C) because both heat and ethanol cause membrane disordering and protein denaturation [29,31,32]. Moreover, some of the ingredients of the ethanol production medium may also disrupt these cells, especially at higher ethanol concentration and elevated temperature.

2. Ethanol Production in Static Cultivation Using 20% (v/v) WBFB Sediment in Distilled Water

In our previous studies [5], the original WBFB (a liquid with suspended/sediment particles) was found to be superior to the supernatant in terms of bio-ethanol production in a shorter time. The concentration of ethanol at 30 °C after 7 days was found to reach the maximum level of 105 g/L in shaking conditions and 91.5 g/L in static conditions using the WBFB supernatant. Similarly, a maximum of approximately 126 g/L of ethanol was produced at 100 rpm using WBFB supernatant contained in a jar fermenter at a temperature raised from 25 to 67 °C in 1 h [5]. However, when the whole original WBFB was used for ethanol production, then the ethanol concentration increased from 45.3 g/L to 222 g/L after one day of cultivation at 33 °C, to 227 g/L after four days and to 258.2 g/L after seven days [5]. These results clearly indicated the role of the solid residue (WBFB sediment), mainly consisting of yeast cells, in bio-ethanol production. To check the feasibility of bio-ethanol production from the solid residue alone, the WBFB sediment was suspended in distilled water and was evaluated for the production of bio-ethanol. The solid residue in WBFB generally ranges between 20 and 30%; therefore, 20% (v/v) solid residue was used in these investigations. The results have been compiled in Fig. 5, which shows the concentrations of ethanol and glucose during the course of fermentation of 20% (v/v) sediments (obtained from WBFB samples with various stock times) suspended in distilled water for 2 h at 67 °C (increased from room temperature). The profile for the increase in temperature is given in Fig. 1. The results for sediments obtained from fresh WBFB (stock time 0 h) indicated an increase in the concentration of bio-ethanol from 1.34% at 0 h to 1.73% after 1 h, followed by a decrease. The decrease in ethanol concentration may be due to its conversion into other chemical entities in the biochemical reactions that occur in the presence of different enzymes produced by yeast, as reported previously [33]. The results for the sediment obtained from five days old WBFB indicate an almost similar trend in bio-ethanol production (Fig. 5). In this case, the ethanol concentration increased from the initial 1.34% to 1.87% after 1 h and thereafter remained constant for the first 2 h of the fermentation period. Almost similar results were obtained for bio-ethanol production from sediment obtained from seven days old WBFB (Fig. 5). In this case, the ethanol concentration increased from the initial 1.38% to 1.75% in 1 h and then slightly decreased to 1.67% after 2 h, probably due to conversion to some other molecules as reported elsewhere [33]. The whole WBFB contains various enzymes derived from malted barley during the beer fermentation process [20,21] which are suitable for the saccharification process [5]. The majority of these enzymes seem to be present in the supernatant because the yeast strains expected to be present in the WBFB sediment [5] cannot produce saccharification enzymes [22]. However, the sediment mixture may contain some quantity of these enzymes.

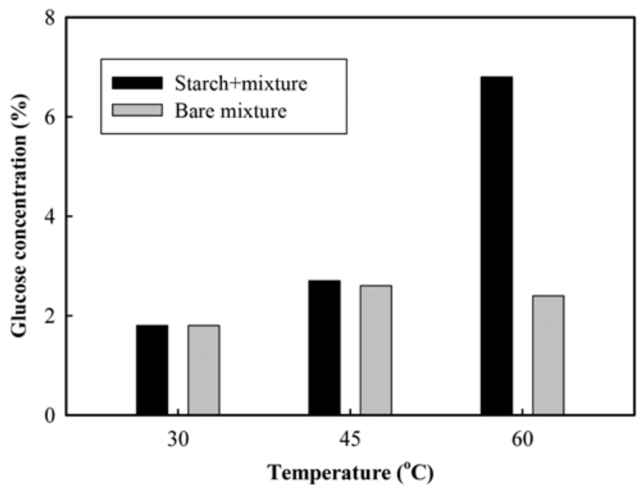


Fig. 7. The concentration profiles of glucose in the 20% mixture of WBFB sediments in distilled water containing 5% additional soluble starch and the bare 20% mixture of WBFB sediments in distilled water during cultivation in a shaking incubator at 150 rpm for 24 h. The initial concentration of glucose in the mixture was 1.5%.

Moreover, the supernatant from WBFB contains high amounts of carbon, nitrogen [16] and other substances including enzymes and yeast cells [5]. The possible reason for the lower bio-ethanol production is that the sediments were suspended in distilled water, which lacks important enzymes and nutrients present in the WBFB supernatant that take part in the saccharification and fermentation processes during bio-ethanol production [5]. Some quantity of such enzymes may be present in the sediment mixture, but these are five-fold diluted in distilled water, which further reduces their concentration. Such enzymes may convert the polysaccharides present in WBFB sediments to simple sugars which accelerates the process of ethanol production. The presence of residual saccharification activity in WBFB sediments is evident from Fig. 7. There is no readily available carbon source in distilled water, unlike in the WBFB supernatant, from which the live yeast cells (if any) can produce bio-ethanol [16]. Moreover, the nutrients present in the WBFB sediments are also five-fold diluted. This can be confirmed by the presence of only a small concentration of glucose in the culture broths of WBFB sediments in distilled water (Fig. 5).

3. Ethanol Production in Static Cultivation Using 20% (v/v) WBFB Sediment in a Chemically-defined Medium

In our previous studies [5], some live yeast cells were found in the WBFB which may be able to produce ethanol if they are supplied with proper nutrients. Therefore, experiments were carried out using 20% (v/v) WBFB sediment in a chemically-defined ethanol producing medium to investigate its ability to produce ethanol in this medium. Fig. 6 shows the results for sediments obtained from WBFB after 1, 2 and 3 days of stocking. The results reveal that there was a 2.09% increase in ethanol production after 2 h of fermentation for sediment obtained from WBFB after one day of stocking. In this case, the ethanol concentration in the medium increased from the initial 1.12% to 3.21% after 2 h of fermentation. The increase in this case is much higher than that obtained for 20% (v/v) sediment in distilled water. These results confirmed that the yeast cells or fermentation enzymes present in WBFB sediments are capable of producing some ethanol from the nutrients, particularly the main carbon source, glucose, which are present in the medium. Interesting results were obtained for the ethanol production from 20% (v/v) sediment in a defined-medium obtained from WBFB with a longer stock time. The results for the sediments obtained from two days old WBFB showed that the ethanol concentration increased from the initial 1.15% to 3.60% in 30 min and decreased thereafter (Fig. 6). This decrease may be due to conversion of ethanol into other chemical entities in biochemical reactions that occur in the presence of the different enzymes produced by yeast [33]. Similarly, ethanol production was even greater for sediments obtained from a WBFB that had been stocked for three days (Fig. 6). In this case, a consistent increase was observed from the initial 1.16%, with the concentration reaching 4.41% after 2 h of cultivation. In our previous investigations [5], bio-ethanol production increased consistently with the stock time of the WBFB. In the current study, it was revealed that there is no appreciable difference in the initial concentration of ethanol produced from WBFB sediments with different stock times (Fig. 6). However, much higher ethanol was produced after fermentation at 67 °C from the older WBFB sediments. The possible reason for this increase in ethanol production with stock time could be that various cell wall hydrolyzing enzymes including endo β-1,4-glucanase, endo β-1,3-glucanase and β-glucan solubilase that are present in the WBFB supernatant [5,20,21] act on the sediment for a longer time. This enzymatic action on yeast cells may loosen or partially remove their cell walls in a similar way to that reported for Zymolyase 20T (an enzyme complex with endo-(1→3)-β-D-glucan hydrolase activity) [34], thereby enabling the fermentation enzymes to rush out on heat treatment during fermentation. These enzymes can in turn lead to the production of bio-ethanol, an assumption well supported by the well-known cell-free fermentation theory of the German chemist Eduard Buchner [35,36]. The decrease in the glucose concentration in the medium further confirms this hypothesis. In all cases, the glucose concentration in the medium decreased (or increased) according to the increase or decrease in ethanol concentration with fermentation time. This decrease (or increase) in glucose concentration was inversely proportional to the increase (or decrease) in ethanol concentration. This ethanol production may be carried out either by living yeast cells using the glucose present in the culture broth or by the enzymes released from the dead cells into the medium (cell-free fermentation) or both. The enzymes may act on the glucose present in the culture broth and convert it to bio-ethanol. The deterioration of yeast cells in the case of a fermentation broth of 20% WBFB sediments in a chemically-defined medium is clearly visible in the microscopic pictures (Fig. 3), which also partially supports the above discussion. The glucose present in the culture broth may come either from the glucose present in the chemically-defined medium or the new glucose converted from the starch that remained in the sediment by the action of the saccharification enzymes. The existence of the extra glucose (other than the medium component) can also be confirmed by the mass balance equation as the quantity of ethanol produced is higher than the quantity of glucose consumed (Fig. 6).

4. Saccharification Activity in Sediments

Saccharification, the process of breaking a complex carbohydrate (such as starch or cellulose) into simple monosaccharide components, is an important step during beer fermentation. This process is usually carried out by various starch hydrolyzing enzymes such as *α-amylase*, *β-amylase* and *limitdextrinase*. Similarly, protein hydrolyzing enzymes, i.e. *endopeptidase* and *carboxypeptidase*, and cell wall hydrolyzing enzymes, i.e., *endo β-1,4-glucanase*, *endo β-1,3-glucanase* and *β-glucan solubilase*, are also produced from malt in the initial stage of the beer fermentation process [20,21]. After beer fermentation, these enzymes may remain in the WBFB and hydrolyze the starch residue present in WBFB during ethanol production. The bio-ethanol production in the present study was carried out at 70 °C (increased from room temperature as shown in Fig. 1). This higher temperature may destroy the activity of many enzymes. According to the literature [37], most of the starch-saccharifying enzymes do not lose their activities even at temperatures as high as 60-90 °C. Previous investigations [5] confirmed the presence of starch hydrolyzing enzymes in the supernatant of the WBFB. The presence of such enzymes in the supernatant, however, does not guarantee their existence in the sediments. And the lower yields of bio-ethanol and lower concentration of glucose in the culture broth obtained in the current study raised suspicions over the presence of these enzymes in sufficient concentrations in the sediments of WBFB, or more exactly in the culture broth. Therefore, experiments were performed to check the existence of starch-hydrolyzing enzymes in the mixture of 20% sediments in distilled water. For this purpose, attempts were made to produce glucose from soluble starch dissolved in the mixture. The concentration of glucose produced in the mixture containing additional soluble starch was not much higher than that in the bare mixture after 24 h of incubation at 30 and 45 °C (Fig. 7). However, this difference was more prominent at 60 °C. Previously, it was found that glucose production is faster at higher temperatures and also in the presence of additional starch [5]. These results show that there is some residual activity of starch hydrolyzing enzymes because the glucose concentration increased with incubation for 24 h especially at 60 °C as shown in Fig. 7. These results are in agreement with previously reported observations of the optimum temperature for saccharification enzymes from malt, which is higher than 50 °C [20,38]. Although some residual enzyme activity was found in the sediment, it was much lower than that in the supernatant alone or in the whole WBFB (supernatant with sediments) as reported earlier [5]. This lower residual enzyme activity may be due to the five-fold dilution of the WBFB sediments in distilled water. Similarly, the trends for ethanol production obtained in the current (Fig. 5 and Fig. 6) and previous investigations [5] also varied according to the extent of the residual activity of starch hydrolyzing enzymes.

CONCLUSION

The results of this study indicated that the freeze-dried sediments are flake-type with porous surfaces and no visible yeast or bacterial cells. The population of yeast cells in a culture broth of sediments in distilled water remained almost constant with no appreciable change in their physical structures. The wear and tear on the yeast cells in a culture broth of sediments in a chemically-defined medium was probably due to higher ethanol concentration and elevated temperature, although their number remained constant. There was a relatively small increase in ethanol production with fermentation and stock time when using WBFB sediments in distilled water. This may be due to the dilution of saccharification and fermentation enzymes and the nutrients present in sediments. However, a higher bio-ethanol production was obtained with WBFB sediments in a chemically-defined medium, which further increased with stock time. The yeast cells or enzymes present in WBFB sediments may produce ethanol from the nutrients present in the medium and sediments. Some residual activity of starch hydrolyzing enzymes was also found in the sediments. The overall results of the current study revealed that the WBFB sediments alone are capable of bio-ethanol production in spite of their five-fold dilution in distilled water or in the chemically-defined medium.

ACKNOWLEDGEMENT

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (No. 2011-0016965).

REFERENCES

1. J. O'Grady and J. A. Morgan, *Bioprocess Biosyst. Eng.*, **34**, 121 (2011).
2. U. G. Akpan, A. A. Alhakim and U. J. J. Ijah, *Leonardo Electron. J. Pract. Technol.*, **7**, 1 (2008).
3. H.-S. Song and D. Ramkrishna, *Korean J. Chem. Eng.*, **27**, 576 (2010).
4. H. Li, L. Yang, Y.-J. Kim and S.-J. Kim, *Korean J. Chem. Eng.*, **28**, 1085 (2011).
5. J. H. Ha, N. Shah, M. Ul-Islam and J. K. Park, *Enzyme Microb. Technol.*, **49**, 298 (2011).
6. Zs. Kádár, Zs. Szengyel and K. Réczey, *Ind. Crop. Prod.*, **20**, 103 (2004).
7. M. Jeya, Y. W. Zhang, I. W. Kim and J. K. Lee, *Bioresour. Technol.*, **100**, 5155 (2009).
8. S. H. Krishna, K. Prasanthi, G. V. Chowdary and C. Ayyanna, *Process Biochem.*, **33**, 825 (1998).
9. C. Martin, M. Galbe, C. F. Wahlbom, B. Hahn-Hägerdal and L. J. Jönsson, *Enzyme Microb. Technol.*, **31**, 74 (2002).
10. M. Han, Y. Kim, Y. Kim, B. Chung and G.-W. Choi, *Korean J. Chem. Eng.*, **28**, 119 (2011).
11. S. Amin, *Energy Convers. Manage.*, **50**, 1834 (2009).
12. F. Xu, Y.-C. Shi, X. Wu, K. Theerarattananoon, S. Staggenborg and D. Wang, *Bioprocess Biosyst. Eng.*, **34**, 485 (2011).
13. M. Boyle, N. Barron and A. P. McHale, *Biotechnol. Lett.*, **19**, 49 (1997).
14. S. Prasad, A. Singh and H. C. Joshi, *Resour. Conserv. Recycl.*, **50**, 1 (2007).
15. L. Zertuche and R. R. Zall, *Biotechnol. Bioeng.*, **24**, 57 (1982).
16. T. Khan, S. H. Hyun and J. K. Park, *Enzyme Microb. Technol.*, **42**, 89 (2007).
17. J. H. Ha, O. Shehzad, S. Khan, S. Y. Lee and J. W. Park, *Korean J. Chem. Eng.*, **25**, 812 (2008).
18. S. Omer, S. Khan, T. Khan and J. K. Park, *Korean J. Chem. Eng.*, **26**, 1689 (2009).
19. T. Khan and J. K. Park, *Carbohydr. Polym.*, **73**, 438 (2008).
20. K. M. Halanych, *Mol. Phylogenet. Evol.*, **4**, 72 (1995).
21. Y. Lin and S. Tanaka, *Appl. Microbiol. Biotechnol.*, **69**, 627 (2006).
22. Y. Fujita, S. Takahashi, M. Ueda, A. Tanaka, H. Okada, Y. Morikawa, T. Kawaguchi, M. Arai, H. Fukuda and A. Kondo, *Appl. Environ. Microb.*, **680**, 5136 (2002).
23. D. J. Schell, N. D. Hinman, C. E. Wyman and P. J. Werdene, *Appl. Biochem. Biotechnol.*, **24/25**, 287 (1990).
24. M. Z. Alam, N. A. Kabbashi and S. N. I. S. Hussin, *J. Ind. Microbiol. Biotechnol.*, **36**, 801 (2009).
25. M. E. Gómez, J. M. Igartuburu, E. Pando, F. R. Luis and G. Mourente, *J. Agric. Food Chem.*, **52**, 4791 (2004).
26. Z. Salvadó, F. N. Arroyo-López, J. M. Guillamón, G. Salazar, A. Querol and E. Barrio, *Appl. Environ. Microb.*, **77**, 2292 (2011).
27. S. A. Schmidt, T. Tran, P. J. Chambers, M. J. Herderich and I. S. Pretorius, *Aust. N.Z. Wine Ind. J.*, **21**, 24 (2006).
28. L. R. Lynd, H. J. Ahn, G. Anderson, P. Hill, D. S. Kersey and T. Klapatch, *Appl. Biochem. Biotechnol.*, **28/29**, 549 (1991).
29. I. M. Banat, P. Nigam, D. Singh, R. Marchant and A. P. Mchhale, *J. Microbiol. Biotechnol.*, **14**, 809 (1998).
30. D. Lloyd, S. Morell, H. N. Carlsen, H. Degn, P. E. James and C. C. Towlands, *Yeast*, **825** (1993).
31. P. W. Piper, *FEMS Microbiol. Rev.*, **11**, 339 (1993).
32. P. W. Piper, *FEMS Microbiol. Lett.*, **134**, 121 (1995).

33. S. Boubekeur, N. Camougrand, O. Bunoust, M. Rigoult and B. Guerin, *Eur. J. Biochem.*, **268**, 5057 (2001).
34. C. Bauer, V. Herzog and M. F. Bauer, *Microsc. Microanal.*, **7**, 530 (2001).
35. R. E. Kohler, *J. Hist. Biol.*, **5**, 327 (1972).
36. E. J. Allian, *J. Chem. Technol. Biotechnol.*, **82**, 117 (2007).
37. E. Stark and P. A. Tetrault, *J. Bacteriol.*, **62**, 247 (1951).
38. S. Takao, H. Sasaki, K. Kurosawa, M. Tanida and Y. Kamagata, *Agric. Biol. Chem.*, **50**, 1979 (1986).