Investigation of Olive Stones as Lignocellulose Material for Bioethanol Production

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Abstract: In Iran, the area under olive cultivation is growing. With increasing the production of olive a lot of waste cake is produced. Olive stone as a lignocelluloses biomass is a raw material suitable for enzymatic hydrolysis and bioethanol production that now only starchy and sugar plants use. In this study, the olive stones were delignified with sodium hydroxide at 50°C and simultaneous velocity of 250 rpm. It has undergone enzymatic hydrolysis. Various quantities of cellulase in different concentrations of achieved cellulose materials have been used. The findings of the study indicated that, although using more enzymes (80 cc) yields more sugar, the amount of sugar produced will be half of the quantity (40 cc) used, with regard to high cost of enzymes. The velocity of reactions dropped dramatically after 8 h. With an 8-h double cycle of hydrolysis and enzymatic recovery, sugar concentration of about 20 g/l is achieved using 200 g of primary cellulose compounds (100 g per cycle) and 40 cc of enzymes. Thus, with 0.55 cc of alcohol efficiency to 1 g of sugar, 11 cc of alcohol is produced in fermentation.

Key words: olive stone · Enzymatic hydrolysis · Bioethanol · Lignocellulosic compounds

INTRODUCTION

Olive tree (Olea europae L.) is one that has been used in producing oil and table olives for many years. Over 95% of the olive trees are in the Mediterranean region [1]. Outside this region i.e., in Iran, its cultivation has grown in recent years and has reached 50,000 ha based on the latest statistics [17]. As a result, large quantities of fruit will be produced and extracted soon. Based on extraction technology, press, biophase, or triphase decanter, in return for every ton of olive fruit, 200 kg of oil, 110, 400, and 1000 liters of vegetable water, and 400 kg of olive pumace are made, respectively. The olive pumace contains 10-11% skin, 21-23% flesh, 36-54% stone, 5-8% oil, and 25% water, which changes to 22-20% and 30-55% in continuous systems [2, 3]. This cake contains 94.23% organic matter, 33.5% lignin, 20.5% cellulose, and 40.6% hemicellulose [4, 5]. Usage of these organic matters hasn't developed in Iran yet. It is used more to increase olive-garden soil fertility and lower quantities are used to feed livestock [6]. However, olive cake has various uses in different countries. Energy production by burning it alone [4, 7, 8], or with other organic and some mineral substances [2, 9, 10], feeding livestock [6, 11], compost making [5], improving soil conditions [3, 12], producing active carbon [3], biogas production [14-17], and hemicelluloses extraction [8] are some of the cases. Yet this lignocellulosic compound has never been used to produce bioethanol [18]. Lignocelluloses compounds refer to substances that contain cellulose and hemicelluloses of polymers made up of sugar monomers that are converted into ethanol after primary treatments, hydrolysis, and microbial fermentation [19, 20]. Many countries including America, Brazil, China, Taiwan, India, and Thailand have decided to substitute part of their fossil fuels for ethanol in the coming years. Some of the reasons for this are less environmental pollution, energy supply security, higher octane, and the production of fewer green house gases [19, 21]. Up to 10% bioethanol can be added to gasoline to boost its combustion; however, there are some modes of transportation that are able to consume up to 85% ethanol [19]. Today’s technologies use grains (corn and wheat) and sugar plants (sugar cane and beetroot) to produce bioethanol. High demand of bioethanol resulted in the study of other sources of biomass [18, 19].

Some of these compounds are produced in large quantities as by-products in agricultural-based industries and are quite cheap [20]. In a study, Peterson, et al. [22] used rye chaff, colza, and fava beans as lignocelluloses

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resources to produce bioethanol [22]. By pre-treating an aquatic oxidation, they could produce 66%, 70%, and 52% alcohol out of rye, colza, and faba beans, respectively. Adrados et al. [23] could also produce ethanol by using wheat bran that comprises 19% of the grain weight (1). Gnanaspreet et al. [20] suggested that sorghum bagasse is more suitable for producing bioethanol than burning it (14). In an experiment, Silverstein, et al. [24] compared different methods of pre-treatment to enhance scariﬁcation of cotton stalks (27). In the near future, bioethanol will be produced by using lignocelluloses compounds such as hard woods, soft woods, and herbaeaceus materials. Movagharnejad et al. [25] have suggested models for enzymatic hydrolysis in heterogeneous liquid-soiled systems (21, 22). This study aims to analyze enzymatic hydrolysis of cellulose stock in olive stone and bioethanol production. Such study has had no place in Iran because of the abundance of oil and gas. However, this will be appreciated soon because of the elimination of petrol subsidies. Applying mathematical models for shrinking particles based on the data gathered in this study can be used to make an accurate prediction regarding the production of sugar during the hydrolysis and fermentation processes.

MATERIALS AND METHODS

Applied Materials: Olive waste cake provided by the Ganje factory in Roodbar (north of Iran) was used to perform this experiment. Olive waste cake usually contains non-cellulose parts that should be separated. After separating the skin and flesh, the stone of the olive was ground and kept in a mortar at 90°C for 1 day. The soiled materials (compounds) were dried and prepared for the experiment [26]. The enzyme used was celloblast (22.85-4) and was supplied by Danish Novo. The enzymatic activity of celloblast was determined to be 42 fpm/ml [27].

Experimentation: A total of 100 g of oven-dried compound was added into 1 liter of sodium hydroxide solution (5 g/l). The resulting compound was boiled at 100°C for 1 h. Large quantities of lignin were obtained from the produced substances and prepared for enzymatic hydrolysis. Through this process, the mixture was stirred well to avoid settlement of the solid particles. Then, the compound was cooled for 1 h and the modified solid particles were separated. The solid particles were then leached to eliminate any alkaline substances and turned into neutral pH by distilled water. After that, the modified solid particles (deligninized) were redried in the oven at 90°C for 1 day.

Enzymatic Hydrolysis: In this experiment, the considered quantities of the achieved cellulose compounds were added into the reaction container and 500 cc of buffer acetate (pH=5) was added. Care should be taken when the solid particles are added to the reaction container to stop them from sticking to the walls of the container. Then the container of cellulose compounds was kept in water bath at 50°C. The temperature of the reaction vessel is kept at 50°C during the hydrolysis process. After fixing the vessel temperature, the mechanical mixer is put in the reaction vessel and the velocity is set at 250 rpm to keep the cellulose particles suspended (22). Following this, the necessary amount of enzymes is added to the compound. The addition of enzymes is the starting point of the process. At intervals of 1 h, 5-ml samples are taken out of the reaction container and added into the test tube and it is immediately put in the boiling vessel for 15 min to prevent any remaining enzymatic activity in the samples. After being cooled, the solid particles are separated by filter paper and the remaining solution is kept until the concentration of the produced sugar is measured. The experiments were over up to the time that the process velocity became too slow. The sugar level of the experiment samples was measured after dilution with dinitrosalicylic acid using the standard method [28, 29]. The primary experiments indicate that when the concentration of the primary substances exceeds 8-10% w/w (80-100 g/L), it may lead to enhanced mass transfer resistances and may block the enzymes from reaching the reactive spot on the outer surface of the cellular particles. Because of this, cellulose compounds at 3 levels of 60, 80, and 100 gr/L concentration were used in the experiments. The enzyme was also used at 4 levels of 10, 20, 40, and 80 ml/L.

Compound Recovery Test: This experiment is in fact the continuation of the previous hydrolysis analysis and is done to absorb part of the free remaining enzymes in the solution container. After an 8-h period, using hydrolysis based on the applied methods in the test, the hydrolysis mixture contents were separated using a filter and the solid part was discarded. The necessary quantities of cellulose compounds were added into the remaining solution and the enzymatic hydrolysis process was continued for another 8-h. This action results in significant progress in the process of cellulose enzymatic hydrolysis. This study has been done as a factorial experiment in a completely randomized design with three replications and the comparison of means was conducted by Duncan test.
RESULTS AND DISCUSSION

The produced sugar was different in various concentrations of cellulose compounds and the highest sugar level was in 100 g/l. There was also a significant difference between used enzymes concentration and the highest produced sugar observed in 80 cc (Fig. 3). By passing time, the produced sugar concentration has increased, however, after 8 h, the speed of the reaction slowed and the trials inevitably stopped (Fig. 3). Although, in laboratory scales, it is possible to continue any experiment up to the desired time but time is important in practical processes and it is undesirable to continue the reaction at a low speed. Usually achievement to the highest level of final product is the goal of chemical processes. In such processes, the cost of catalyst versus the cost of primary materials, production expenses, or the cost of the final product are dispensable. However, in hydrolysis trials, the main costs are related to the cost of enzymes and the process is more economical if fewer enzymes are used. On the other hand, ethyl alcohol purification is one of the major costs of the project, so it is necessary to produce more sugar in the solution. This issue has been ignored in most of the conducted research and process efficiency has been defined as the produced glucose over the utilized primary materials. According to the results obtained by these researchers, optimum process efficiency is achieved when higher quantities of enzymes are added to the primary materials. However, due to the high costs of enzymes utilized, it is not economical. Therefore, the interaction effects of the applied treatments in the trials must be noted.

The trial results have shown that although the highest level of sugar concentration has been obtained in 100 g/l of cellulose compound concentration with the presence of 80 cc enzyme, there has been no significant difference with 80 g concentration and 40 cc enzyme (Fig. 2). Even good results were achieved by using 20 cc of enzymes in a 60 g/l concentration of cellulose compounds (Fig. 1). In all utilized concentrations of the cellulose compounds, the obtained sugar reached its peak after 8 h and the speed of reaction slowed down despite of achieving the maximum sugar concentration in the presence of 100 g/l cellulose compounds. However, it seems that the resulting sugars are desirable 6 or 7 h after the trial as well, and is more than the concentration of 80 g in 8 h. Provided that the time factor is considered important in such processes, it is possible to mark this result as practical.

The interactions of the utilized treatment have indicated that the maximum level of sugar is obtained after 8 h with 100 g/l concentration of cellulose compounds and with the presence of 80 cc of enzymes, but the sugar produced after 7 h (even 6 h) is also acceptable. At the same concentration of cellulose compounds, when the used enzyme is halved, the acceptable sugar is produced after 8 h. At lower concentrations of cellulose compounds, using more enzymes does not result in more sugar.

To achieve a higher concentration of sugar solution and to economize using enzymes, it is necessary to utilize a higher concentration of the primary materials and to reuse the enzymes.

Several cycles of enzyme recovery were tested. The results showed that the best practical method to recover enzymes is to apply fresh primary materials. The study results have demonstrated that using a 100 g/l concentration of cellulose compounds with 40 cc of enzymes (42 fpu) and conducting one recovery operation in two cycles of 8-h with the same amount of enzymes provides the best results. In other words, two 8-h cycles of hydrolysis and enzyme recovery with 200 g of

![Fig. 1: The produced sugar for 60 g/l cellulose compounds and the different amounts of enzyme](image_url)
primary materials prepared (100 g per cycle) and 40 cc of enzymes provides 20 g/l of sugar concentration (2%). Since it is possible to produce about 55% alcohol volume out of 1% sugar in fermentation, it is concluded that it is possible to produce approximately 11 cc of alcohol out of every 200 g primary materials.

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REFERENCES


