THE USAGE OF CARROT POMACE FOR BIOETHANOL PRODUCTION

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ABSTRACT

The lignocellulosic substances such as agricultural wastes are promising feedstocks for bioethanol production. Because they are cost effective, renewable, abundant and not having primary value for food and feed. The current study suggests that improvements in polysaccharide hydrolysis of under-utilized biomass of carrot pomace may find practical use in its conversion to bioethanol by *Saccharomyces cerevisiae* and *Pichia stipitis* fermentation. Some important parameters for bioethanol production such as pretreatment procedures (CaO and activated charcoal treatments), nitrogen sources ($(NH_4)_2SO_4$, soy wheat, cheese whey), and pomace loading amount (15-120 g/L) were optimized in the study. The highest bioethanol production could be achived when the saccharification and fermentation conditions were optimized in order to increase monosaccharide yield and fermentation of both six-carbon and five-carbon monosaccharides. The bioethanol production was 1.9 –fold higher for *S. cerevisiae* and 4.6 –fold higher for *P. stipitis* when $(NH_4)_2SO_4$ was added in addition to the trace nitrogen substances, vitamins and minerals present in carrot pomace. The highest bioethanol production values were obtained as 6.91 and 2.66 g/L in the presence of 120 g/L pomace loading, 1 g/L $(NH)_2SO_4$ at the end of 72 hours incubation time at pH 6 by *S. cerevisiae* and *P. stipitis*, respectively.

Keywords: bioethanol; agricultural waste; yeast; fermentation; carrot.

INTRODUCTION

In today's world, there is an urgent need for alternative energy sources due to rapid depletion of fossil fuels and environmental concerns. Thus a lot of studies are performed to develop new technologies from clean, safe and renewable sources. Of these biomass is a good candidate instead of fossil fuels because of its environmental friendly properties. It has been estimated that among the fuels obtained from biomass, bioethanol will be the most widely used renewable source in the near future as ethanol can also be used as an energy source in industry or as a fuel for vehicles.[1,2] Because feedstock costs are major part of the bioethanol production, considerable work has been performed toward production of bioethanol using various kinds of feedstocks such as starch rich agricultural wastes and cellulosic biomass.[3] The usage of lignocellulosic biomass (wood, grass, agricultural wastes, munipicipal solid wastes etc.) for bioethanol production has some advantages such as having abundant carbonhydrate content and fermentable sugars, being cheaper, having nearly zero greenhouse gas emission and not derived from a food source.[4,5] There are considerable work about bioethanol production from lignocellulosic biomass and agricultural wastes by fermentation in the literature.[6-10]

As fruit juices are among the most popular non-alcoholic beverages, their pomaces present a serious disposal challenge and are considered as under-utilized biomass. Among them carrot pomace is suggested as one of the excellent substrates for bio-processes, as a rich source of soluble and insoluble carbohydrates. Another important point is which directed us to use of carrot pomace as a feedstock for bioethanol production is it being one of the few plants that accumulate free sugars into vacuoles as reserve. Furthermore valuable compounds such as carotenes, uronic acids, natural sugars and several minerals are still retained in carrot pomace after processing. Thus these nutritional properties of carrot pomace would allow preperation of a suitable growth and fermentation medium for the yeast cells to produce bioethanol.[11-13] On the other hand the wastes of food processing pose increasing disposal and potential severe disposal problems. Therefore in the current study it was aimed to use valuable biomass and nutrients obtained from carrot pomaces as feedstocks for bioethanol production while decreasing the environmental load. The goal of the study was to develop a simple bioprocess of bioethanol production from fruit wastes. *Saccharomyces cerevisiae* and *Pichia stipitis* yeasts were used for the fermentation process. Some important parameters for bioethanol production such as feedstock preperation, pomace loading and nitrogen sources in the fermentation medium were optimized to find the highest bioethanol production from the fermentation of both yeast cells.

Although extensive research work has been carried out on usage of agricultural wastes as raw materials for bioethanol production, to our knowledge this is the first report about practical usage of carrot pomace for bioethanol production.

EXPERIMENTAL

Raw Material Preperation

Carrot pomaces (CP) were obtained from a local company (BELSO) in Ankara-Turkey. The carrot waste was collected after juice extraction and stored at -20 ^{0}C until use. Different ratios of pomace were used for hydrolysis and fermentation.

Hydrolysis of Raw Material

For the hydrolysis studies, a weighed amount of carrot pomace was suspended in 100 ml of distilled water and homogenized in a homogenizer (Thermo Scientific-USA) at 8000 rpm for 10 min. Then 1.5% H_2SO_4 (v/v) (Sigma-Aldrich USA) was added to the pomace and boiled for 10 minutes. After hydrolyzing, the mixture was filtered and the filtrate that contained pomace sugar was used for detoxification experiments.

Detoxification of Raw Material

To eliminate the fermentation inhibitors, the filtrate was treated with calcium oxide and activated charcoal. The pH of the filtrate was increased to 10 by adding calcium oxide and decreased to 6 with H_2SO_4. By this way the insoluble compounds were filtered and the medium were centrifuged at 5000 rpm for 10 min. The supernatant obtained after centrifugation was filtered again. The detoxified filtrate was autoclaved at 121 ^{0}C for 15 min and was used for the fermentation experiments. The calcium oxide and activated charcoal was purchased from Merck. The CAS numbers of activated charcoal and calcium oxide are 7440-44-0 and 1305-78-8, respectively.

Microorganisms

Saccharomyces cerevisiae and *Pichia stipitis* yeasts were used for fermentation process. *S. cerevisiae* was obtained from Ankara University, Faculty of Science Laboratories' from the current culture collection. *P. stipitis* was obtained from ARS (NRRL) culture collection.

Fermentation conditions

To see the effects of biomass loading on bioethanol production, increasing carrot pomace loading concentrations from 15 g/L to 120 g/L were tested.

The effects of different nitrogen sources on bioethanol production were also investigated in the study. For this purpose, cheese whey (1 g/L), soy wheat (1g/L), and $(NH_4)_2SO_4$ (1 g/L) were used as nitrogen sources in the medium prepeared with pomace sugar.

The yeast cells were precultured in the medium which was containing carrot pomace sugar as a carbon source at 30 ^{0}C for 72 hour in a rotary shaker at pH 6. 10% yeast suspension was aseptically transferred to 5-ml anaerobic fermentation medium which was prepeared by carrot pomace sugar containing distilled water at pH 6. The sugar concentration and the microbial growth were monitored periodically throughtout the fermentation. Each of the experiments and the measurments described were performed in triplicate.

Analytical Methods

The bioethanol concentration was analyzed using gas chromatography (Shimadzu, Model GC-14B) equipped with a flame ionization detector. One microlitre of sample was injected through a glass column (2 mm i.d., 2 m long)

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packed with chromosorb 101 (80/100 mesh). The injection port and flame ionization detector temperatures were held at 220 and 250 °C, respectively. Oven temperature was kept constant at 160 °C and nitrogen was used as carrier gas.[14]

The growth of yeast cells was determined by measuring optical density at 600 nm wavelengths for the measurement of microbial growth during the incubation period. A standard calibration curve for yeast concentration was constructed based on the dry weight and OD600 measured from the yeast culture.[15] The sugar concentrations were determined according to the phenol-sulfuric acid method.[16]

Absorbance measurements and centrifugation were performed using a Shimadzu UV 2001 model spectrophotometer and Hettich EBA12 model centrifuge, respectively.[17]

RESULTS AND DISCUSSION

The carrot pomace was used as a feedstock for bioethanol production. The parameters such as pretreatment procedures, biomass loading and nitrogen sources in the fermentation medium were optimized to find the highest bioethanol production by *S. cerevisiae* and *P. stipitis* yeasts.

Pretreatment of Carrot Pomace

The most important step in biofuel production from lignocellulosic biomass is to optimize the pretreatment processes.[18] Pretreatment methods are either physical or chemical or some of incorporate both of them. Acids or bases that promote hydrolysis and improve the yield of fermentable sugars during pretreatment. The most commonly used acid and base are H_2SO_4 and NaOH, respectively.[19] Pretreatment of biomass in autoclave is a common method for lignocellulosic feedstock for bioethanol production. For example, in a study that is performed for bioethanol production from sugarcane trash, pretreatment was carried out in a laboratory autoclave at 121 °C for 60 min.[20] In another study that is about usage of grape marc as a source for bioethanol production, the researchers reported that the acid/autoclave treatment liberated the highest proportion of monosaccharides for both red and white marc and the dilute acid (0.5 M) at higher temperature and pressure was the most effective pre-treatment.[21] Therefore physicochemical treatment method (acid hydrolysis in autoclave) which is a common and environmentally friendly approach was used for pretreatment of carrot pomace in the current study. For this purpose the biomass was autoclaved at 121 ºC for 15 minutes in the presence of 1.5% H_2SO_4. The resulted hydrolyzed starch solution was neutralized with NaOH and prepared as a fermentation medium for the yeast cells. However during the hydrolysis a wide range of inhibitors could be released. It is important to remove these inhibitory compounds from hydrolysate for the fermentation process.[22] Overliming of hydrolysate renders efficient removal of some toxic compounds with a reasonable cost.[23] The activated charcoal treatment and calcium oxide treatment are among in several methods that have been developed for eliminating these inhibitory compounds. pH adjustment with alkali agents like NaOH or $Ca(OH)_2$ can cause precipitation of the toxic compounds.[24] After pH adjustment, toxic compounds can be adsorbed by the activated carbon. In this context activated charcoal and calcium oxide was used for detoxification experiments in the current study. Figure 1 depicts the effects of detoxification methods on growth of *S. cerevisiae.* The date shows that there was no significant difference between activated charcoal treated carrot pomace and not treated carrot pomace. On the other hand the highest cell growth was obtained as 0.067 g/L in the medium treated with calcium oxide.

The Effect of Carrot Pomace Loading on Microbial Growth

It is known that during the bioethanol production process various parameters including the amount of substrate loading significantly influence the total amount of released fermentable sugars. For this purpose to see the effects of biomass loading on bioethanol production, increasing CP concentrations from 15 g/L to 120 g/L were investigated. The effects of variation of CP loading on sugar concentration and *S. cerevisiae* yeast growth were shown in Table 1. The table depicts that, a significant increase in sugar consumption and yeast growth was observed when the biomass increased from 15 g/L to 30 g/L. Higher values for yeast growth were obtained in the presence of higher pomace concentration. More sugars in the fermentation medium caused higher yeast growth. The consumed sugar concentrations were close to each other at 60 and 120 g/L pomace concentrations. The substrate and initial sugar concentrations that used in the current study are compatible with the literature. For example in a study about usage of carrot and yeast discards for the obtention of ethanol, the researchers tested increasing amount of initial sugar concentrations of carrot juice from 0 to 100 g/L. They found out that at nearly 30 g/L initial sugar concentration approximately 7 g/L ethanol concentration was observed.[11] In another study about ethanol production from corn, potato peel waste, researchers stated that substrate concentrations of 5,10 and 15% were used for the production of ethanol, out of which 10% gave the highest yield.[25] Consistent with the literature and because of the highest yeast growth and consumed sugar were obtained in the media prepeared with 120 g/L CP as 0.067 g/L and 16.14 g/L, respectively, the next experiments were performed with this biomass loading concentration.

Table 1. The effect of CP loading on microbial growth (fermentation pH:6, fermentation time: 72 h)

CP loading (g/l)	Sugar concentration (g/l) Initial	Consumed	Yeast growth (g/l)
15	5.69 ±0.48	3.98 ±0.14	0.027 ±0.001
30	13.22 ±0.44	10.02 ±0.53	0.049 ±0.003
60	23.04 ±1.08	15.31 ±0.74	0.055 ±0.003
120	31.76 ±1.16	16.14 ±1.37	0.067 ±0.005

Table 2. The effect of different nitrogen sources (1 g/l) on bioethanol production of *S. cerevisiae* (120 g/l CP, fermentation pH: 6, fermentation time: 72 h)

Nitrogen source (1 g/l)	Bioethanol production (g/l)	Yeast growth (g/l)
Soy wheat	4.77 ±0.13	0.120±0.01
Cheese whey	3.05 ±0.33	0.170±0.02
$(NH_4)_2SO_4$	6.91 ±0.12	0.216±0.02
Control (Only CP)	3.61 ±0.76	0.098±0.01

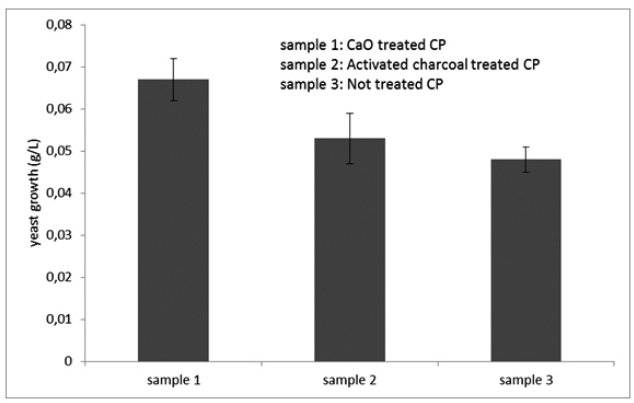

Figure 1. Comparative analysis of *S. cerevisiae* growth in through fermentation in treated with calcium oxide, activated charcoal and without any treatment carrot pomace (biomass lading: 120 g/L CP, 72 hours of incubation time, fermentation pH:6)

The Effect of Nitrogen Sources on Bioethanol Production

Nitrogen sources have crucial importance on the fermentation rate directly. Because nitrogen is a very important nutrient for growth and metabolism of yeast cells. It has been showed that ammonium sulfate and nitrates are the most popular nitrogen nutrition used in sugar-based industrial scale fuel ethanol processes.[26-28]

The usage of carrot pomace as a feedstock is a new approach for bioethanol production. Therefore there are very limited studies about it in the literature. For example in a study which was performed by using carrot juice instead of pomace as a raw material for ethanol production, bioethanol concentration obtained in the presence of 30 g/L initial sugar was very close to each other with our current study which was performed with pomace of carrot. Their study shows that there was no variation in fermentation yield with increasing substrate concentrations and ensure that sugar and ethanol inhibition do not limit the ethanol yields. Similarly we obtained increasing yeast growth in the presence of increasing carrot pomace.[11]

Three different nitrogen sources (soy wheat, whey and $(NH_4)_2SO_4$) were tested in the medium containing carrot pomace sugar as a carbon source to investigate the effect of nitrogen type on the bioethanol production of *S.*

cerevisiae and *P. stipitis*. The initial nitrogen concentration was 1.0 g/L for all of the tried nitrogen sources. The data in Table 2 depicts the different nitrogen sources on bioethanol production of *S. cerevisiae* yeast. In the media supplemented with $(NH_4)_2SO_4$, the initial sugar concentration was 32.8 g/L and after 72 hours of incubation time the *S. cerevisiae* cells consumed 22.6 g/L sugar to produce 6.91 g/L bioethanol. It means the cells converted 21% sugar to bioethanol. On the other hand in the control media which does not contain any additional nitrogen source, the initial sugar concentration was 32.0 g/L and after the same incubation time the cells consumed 12.0 g/L sugar to produce 3.61 g/L bioethanol. So the cells converted 11.3% of the initial sugars. It means that bioethanol production is 1.9 –fold higher when $(NH_4)_2SO_4$ was added in addition to the trace nitrogen substances, vitamins and minerals present in carrot pomace. The data in Table 3 shows that the same trend was also obtained for *P. stipitis* cells. The highest bioethanol production was 2.66 g/L in the media containing 1g/L $(NH_4)_2SO_4$. In the current study to seek of cheap and accessible nitrogen source for fuel ethanol production, soy wheat and whey were also tried as nitrogen sources. However both of the yeast cells showed higher bioethanol production in the media supplemented with inorganic nitrogen source.

Table 3. The effect of different nitrogen sources (1 g/l) on bioethanol production of *P. stipitis* yeasts (120 g/l CP, fermentation pH: 6, fermentation time: 72 h)

Nitrogen source (1 g/l)	Bioethanol production (g/l)	Yeast growth (g/l)
Soy wheat	0.68 ±0.18	0.083±0.04
Cheese whey	1.49 ± 0.04	0.052±0.03
$(NH_4)_2SO_4$	2.66 ± 0.53	0.131±0.07
Control (Only CP)	0.58 ± 0.07	0.062±0.01

CONCLUSIONS

This study demonstrated that carrot pomace is an attractive biomass for bioethanol production. The highest bioethanol production could be achived when the saccharification and fermentation conditions were optimized in order to increase monosaccharide yield and fermentation of both six-carbon and five-carbon monosaccharides as 6.91 and 2.66 g/L by *S. cerevisiae* and *P. stipitis*, respectively. The results obtained from the current study strongly suggest that the biomass of carrot pomace is a promising renewable feedstock for cost- effective second generation bioethanol production.

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