Production and optimization of bioethanol from sour orange (Citrus aurantium) peel using baker's yeast

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Abstract-Usage of fossil fuel has been known to cause adverse effects on the environment due to the emission of harmful greenhouse gases. Production of bioethanol from biodegradable waste material could be an alternative fuel system to the expensive petroleum products. The objective of the study was to determine the suitability of sour orange peel as an effective substrate for bioethanol production using baker's yeast (Saccharomyces cerevisiae). The sour orange peel extract was inoculated with Saccharomyces cerevisiae with the fermentation media composed of 100 g/L sucrose, 5 g/L yeast extract, 10 g/L KH2PO4, 2 g/L (NH4)2SO4, and 0.5 g/L MgSO4·7H2O in order to produce ethanol at room temperature for 24 hours. Ethanol content produced from fermented sour orange peel was measured by an Ebulliometer. Initially the amount of ethanol produced from the orange peel extract was 5% (v/v) at room temperature (30±2 °C) after 24 hours of fermentation. When different inoculum size such as 0.5, 1.0, 1.5 and 2.0 g /100mL were used in the fermentation media, ethanol production was increased to 5.25% when inoculum size of 1.5 g /100mL was used. When different carbon sources such as glucose, maltose, sucrose and lactose were used in the fermentation media, ethanol production was increased to 5.5% in media containing maltose. After the optimization of culture conditions such as fermentation time (24 hour) temperature (37oC) and pH (5.0), the production of ethanol was significantly increased to 6.5% in sour orange peel medium. When different nitrogen source such as ammonium sulphate, ammonium nitrate, ammonium chloride or urea was used in the fermentation media, ethanol production was significantly increased to 7.5% in orange peel media containing ammonium nitrate. The current study concludes that 7.5 % (v/v) ethanol (1.5 times higher) can be produced from sour orange peel using baker's yeast under optimized conditions including maltose as the C source and ammonium nitrate as the N source. Large scale fermentation study should be carried out with bioreactor to determine whether this finding could be commercialized.

Key words: Baker's yeast, Bioethanol, Optimization, Sour orange peel

1.0 INTRODUCTION

The rapidly depleting non-renewable resources has already reached pinnacle. Now there has been a need for a renewable and sustainable energy sources. Ethanol had been a promising renewable source. Bioethanol used for production of gasoline can reduce vehicle carbon dioxide emission by 90% [1]. Ethanol production is usually accomplished by chemical synthesis of petrochemical substrates and microbial conversion of carbohydrates present in agricultural produce. The method of ethanol production from various agro residues is of prime importance as the raw materials are readily available and less expensive. Obtaining high ethanol yield by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology [2].

Sour orange/Bitter orange (Citrus aurantium) is a member of the Rutaceae family, a hybrid between Pummelo and Manderin. This plant is widely grown hybrid in Asia and also available in various tropical countries including Sri Lanka. It is one of the underutilized plants found in Sri Lanka and parts of this plant can be utilized for the production of value added products such as bioethanol, essential oils, citric acid and pectin. Orange peels belong to valuable biomass wastes. The peel contains various carbohydrate polymers, which make it valuable choice for production of metabolites such as ethanol by appropriate microorganisms. There have been no reports published on the usage of the sour orange (Citrus aurantium) for the biofuel production [3]. Therefore this study was aimed to produce bioethanol from peel of sour orange using baker's yeast, characterize the chemical properties and to find ways to improve the yield.

2.0 MATERIAL AND METHOD

2.1 Pretreatment of orange peels

The sour orange (Citrus aurantium) samples were collected from different parts of Jaffna district. Initially sour orange peels were separated from the fruit by using knife and dried. Then the peels were soaked and ground with a food homogenizer to less than 2 mm in diameter. The peels were degraded to convert the cellulose content into more available sugars by dilute acid hydrolysis with little modification to the procedure described by [4].

2.2 Fermentation

Sour orange peel extract medium containing 100 g/L sucrose 5 g/L yeast extract, 10 g/L KH2PO4, 2 g/L (NH4)2SO4, and 0.5 g/L MgSO4·7H2O was prepared. Liquid media was first autoclaved. After autoclaving the conical flask containing 100mL fermentation media was inoculated with 0.5g of Saccharomyces cerevisiae. Each flask was cultured at room temperature under oxygen limiting condition for 24 hr. The oxygen limiting condition was prepared by sealing the flask tightly with parafilm and keeping in an anaerobic chamber [5].

2.3 Physico-chemical characterization of sour orange peel extract

The parameters such as total soluble solids, pH, acidity as citric acid%, and concentration of ethanol were continuously monitored during the fermentation for 4 days at 24 hour interval using refractrometer, pH meter, titration method and ebulliometer respectively [6].

2.4 Effect of inoculum size on bioethanol production

Different inoculum size of yeast such as 0.5g, 1g, 1.5g and 2g was inoculated in the fermentation media to find out the optimum inoculum size to produce maximum amount of bioethanol [7].

2.5 Effect of carbon source on bioethanol production

Initially inoculum size was optimized for the maximum bioethanol production using sucrose as a C source. In this experiment different types of C sources such as glucose, maltose, sucrose and lactose were used to replace sucrose in the fermentation media and the yield of bioethanol was measured at previously optimized conditions. Bioethanol production was determined by ebulliometer [7].

2.6 Effect of incubation period on bioethanol production

The amount of bioethanol production at different incubation times such as 24, 48, 72 & 96 hours was quantified to find out the optimum incubation period for maximum bioethanol production [7].

2.7 Effect of pH on bioethanol production

The pH of the sour orange peel extract medium was adjusted to different level (4.0, 5.0, 6.0, and 7.0) using different concentrations of NaOH to find out the optimum pH for maximum bioethanol production. The yield of ethanol was measured at regular interval by ebuliometer [5].

2.8 Effect of nitrogen sources on bioethanol production

In this experiment different types of nitrogen source such as ammonium sulphate, ammonium nitrate, ammonium chloride, and urea were added to replace the ammonium sulphate in the fermentation media to find out the best N source to produce maximum of amount bioethanol [7].

2.9. Statistical analysis

All the experiments were done in triplicates. Statistical analysis was done by using ANOVA Duncan and Dunnet method were performed to check whether there was any significant difference between the treatments.

3.0 RESULTS AND DISCUSSION

3.1 Optimization of fermentation conditions

Based on the results of sour orange peel extract before the inoculation, orange peel media contained higher amount of total soluble solids and the alcohol content was nil. After fermentation using baker's yeast, the total soluble solids content reduced to 6.8brix, while the alcohol content increased to 5 % (Table 3.1). This was due to the effect of anaerobic fermentation.

Table 3.1: Total soluble solids, pH acidity and alcohol content of sour orange peel extract

Peel	Initial	Final
TSS (Brix)	13.0	6.80
pH	4.61	4.20
Alcohol (%)	0.00	5.00
Citric acid (%)	16.9	15.0

Value represents mean of two replicates

3.2 Effect of inoculum size on total soluble solids, pH, acidity and alcohol content of the sour orange peel

Optimization of inoculum size (0.5g, 1g, 1.5g, 2g) was done to obtain maximum bioethanol production from the sour orange peel extract medium. The results showed that initial total soluble solids of the medium containing orange peel extract was13 brix, and after the fermentation it was reduced to 6 brix (Table 3.2).

Table 3.2: Effect of inoculum size on total soluble solids, pH, acidity and alcohol content of sour orange peel

Peel	Inoculum size							
	0.5g		1g		1.5g		2g	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
TSS (°B)	13	6.	13	6	13	6	13	6
pH	4.9	4.3	4.9	4.3	4.9	4.3	4.9	4.3
Citric acid (%)	14	12	14	11	14	12	14	12
Alcohol (%)	0.0	5.0	0.0	5.0	0.0	5.25	0.0	5.25

Value represents mean of three replicates

Based on the Duncan and Dunnet method ethanol production of peel extract was not significantly between inoculum sizes. Inoculum size of 1.5g and 2 g were given maximum ethanol production of 5.25%. Based on the ethanol yield the inoculum size 1.5g was taken as an optimum level of inoculum.

3.3 Effect of carbon source on total soluble solids, pH, acidity and alcohol content of the sour orange peel

The fermentation was carried out to optimize the carbon source (glucose, maltose, sucrose, lactose) to obtained maximum bioethanol production. The amount of ethanol production was increased to 5.5 % which was added with maltose (Table 3.3).The total soluble solids was reduced to 5.5-6.0 brix for the media containing sucrose, glucose or

maltose as a C source, but there was no significant change in the alcohol yield when lactose was added. The reason may be the yeast unable to utilize the lactose as other carbon source. Based on the Duncan method ethanol production was higher in media with maltose (5.5%) as a C source followed by sucrose, glucose and ethanol production was lowest in lactose. Under the optimized conditions chosen, pH of the media was reduced with the increase in the citric acid percentage. Similar condition facilitated the production of citric acid in yeast [7]. Based on the ethanol yield the maltose was selected as an optimum carbon source for further studies.

Table 3.3: Effect of carbon source on total soluble solids, pH, acidity and alcohol content of the sour orange peel

Peel	Sucrose		Glucose		Maltose		Lactose	
	Initia	Fina	Initia	Fina	Initia	Fina	Initia	Fina
TSS(°B)	12	5.5	11	5.3	12	5.9	12	12
pH	4.7	4.1	4.7	4.2	4.7	4.4	4.7	4.6
Citric acid %	12	20	12	19	11	17	12	14
Alcohol (%)	0.0	5.0	0.0	5.0	0.0	5.5	0.0	0.0

Value represents mean of three replicates

3.4 Effect of fermentation time on total soluble solids, pH, acidity and alcohol content of the sour orange peel

The fermentation time used to produce the ethanol was measured and the results shows that there was no significant change in the production of ethanol in 24hrs, 48hrs, 72hrs and 96hrs (Table 3.4). This may be due to the inadequate nutrition for the yeast to grow.

Based on the Duncan and Dunnet method, ethanol production did not differ significantly between fermentation times. Therefore, 24 h selected as an optimum fermentation time and used for further studies.

Table 3.4: Effect of fermentation time on total soluble solids, pH, acidity and alcohol content of the sour orange peel

Peel	Fermentation time (days)				
	Initial	1	2	3	4
TSS (°B)	12.0	7.0	7.0	7.0	7.0
pH	4.65	4.22	4.24	4.24	4.25
Citric acid (%)	17.28	15.68	15.36	15.36	15.36
Alcohol (%)	0.00	6.00	6.00	6.00	6.00

Value represents mean of two replicates

3.5 Effect of pH on total soluble solids, pH, acidity and alcohol content of the sour orange peel

The results of optimizing the culture conditions such as pH (4.0, 5.0, 6.0 and 7.0) indicated that, the changes in pH could affect the final ethanol concentration. The final ethanol concentration of sour orange peel medium adjusted to different pH showed that medium with pH 5.0gave maximum ethanol yield of 6.5% (Table 3.5). Based on the Duncan and Dunnet method ethanol production was not significantly different between the media with different pH. Based on the ethanol yield, pH 5.0 was selected as an optimum level of pH.

Table 3.5: Effect of pH on total soluble solids, pH and acidity of the sour orange peel

Peel	pH							
	4.0		5.0		6.0		7.0	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
TSS (°B)	12	6.5	11	6.0	10	6.5	11	6.9
pH	3.9	3.9	5.0	5.1	6.0	6.2	6.9	6.8
Citric acid (%)	20	19	11	10	5.4	5.4	0.6	1.2
Alcohol (%)	0.0	6.25	0.0	6.5	0.0	6.0	0.0	6.0

Value represents mean of two replicates

3.6 Effect of nitrogen source on total soluble solids, pH, acidity and alcohol content of the sour orange peel

The final optimization condition was the nitrogen source and when different nitrogen source such as ammonium sulphate, ammonium nitrate, ammonium chloride, and urea were used in the fermentation media, the amount of ethanol production was significantly increased in sour orange peel media (7.5%) with ammonium nitrate (Table 3.6). Therefore, based on the alcohol production with minimum TSS usage NH4NO3 was selected as an optimum nitrogen source.

Table 3.6: Effect of Nitrogen source on total soluble solids, pH and acidity of the sour orange peel

Peel	Ammonium sulphate		Ammonium nitrate		Ammonium chloride		Urea	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
TSS (°B)	14	6.9	14	7.0	14	6.6	14	6.2
pH	4.6	4.2	4.6	4.1	4.6	4.1	4.7	4.1
Citric acid (%)	12.8	18.2	12.8	15.3	14.7	16.0	12.1	14.7
Alcohol (%)	0.00	6.75	0.00	7.5	0.00	6.75	0.00	6.75

Value represents mean of two replicates

4.0 CONCLUSION

The study revealed that the sour orange peel was found to be a very effective substrate for ethanol production. The sugars produced by enzymatic hydrolysis from peels extracted from fruits of sour orange readily converted into ethanol (5% (v/v) by using Saccharomyces cerevisiae through fermentation at 30°C for 24 hours. After the optimization of the culture conditions and the fermentation media, the amount of ethanol production was significantly increased in orange juice by 1.5 times. The current study concludes that 7.5 % (v/v) ethanol can be produced from sour orange peel using baker's yeast under optimized conditions including maltose as the C source and ammonium nitrate as the N source. Large scale fermentation study should be carried out using a bioreactor to determine whether this finding could be commercialized.

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