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Utilization And Selection Of Food Waste As Substrate For Fermentation Using Saccharomyces Cerevisiae.

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ABSTRACT

Food wastes are generated at every nook and corner of the globe on a daily basis, and they poses serious environmental issues. Recycling and different techniques of utilization of these wastes are already available, significantly minimizing food processing waste worldwide. The current study aims at utilizing a few of the food wastes that are generated locally on a daily basis, and dumped on the streets without any utilization of numerous nutritional components. Many of these components may have potential as substrates for production of microbial metabolites e.g. ethanol. Analysis shows that these wastes can be extracted, under specific conditions, for reducing sugar –the basic nutritional component for growth of Saccharomyces cerevisiae, one of the commonly used ethanol fermenting yeasts. In this research work, optimization of anaerobic fermentation conditions were achieved to improve ethanol yield. Among the three available substrate selected, the 30 minute extract of Bagasse gives the maximum reducing sugar content and on estimation for alcohol it also gives the best result (0.202% w/v) than Spent Tea Extract and Hydrolyzed Potato Wash Water. Although the wastes used here are not completely degraded but they possess potential for utilization in ethanol production, which forms one of the industrially important products.

Keywords: Food waste, Fermentation, Saccharomyces, Alcohol

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INTRODUCTION

Food and agricultural wastes are generated on a daily basis; they are disposed in the environment, and they contribute to increase in the carbon footprint. According to the Food and Agriculture Organization this also severely affects climate change in the world [1].Food wastes generated locally and in large supermarkets and retail stores also contribute significantly to this problem [2,3].Different categories of food wastes of different origin can pose problems that may create global nuisance and food shortage.[4,5]

Bagasse is one of the solid wastes generated after extraction of sugarcane juice.The lignocellulosic biomass of bagasse were repeatedly used for the production of fuel alcohol either by direct inoculation of culture [6,8,9] or by using immobilized cells to improve performance.[7]Some have adapted a genetically engineered xylose fermenting strain to sugarcane bagasse hydrolysates in presence of certain inhibitors, which are commonly found in food waste. [10] Besides Saccharomyces, some pentose fermenting novel strains were also used for utilization of bagasse waste.[11]

Spent tea were mostly used for the purpose of cell mass growth in solid phase. The enzymatic saccharification process has beenoptimized [12, 14] for different sources of tea or acidic saccharification can also be done. Spent worm tea can itself be used as a pretreatment process for the production of second generation bioethanol.[13]; else fungal biomass grown on spent tea can be used as inoculum for fermentation process. [15]

Raw potato wash water contains less sugar than hydrolyzed one. The saccharification and hydrolysis can be done either by optimized acid treatment[16, 17] or enzymatic reaction with the potato starch[18]. Bioethanol productionhas been one of the major aims of this treatment using overnight soaked wash water [18], waste potato mash [19], soft rotten potato [20], potato tuber [21]or potato peels[22]as substrate. Along with ethanol, some amount of acetone and butanol production was made possible by using integrated membrane extraction after fermentation of the potato waste[23].

In this research work,these three wastes were selected based on their availability, cost,bulk production and ease of processing and handling.Liquid wastes or extract were preferred than solid state fermentation as the latter may involve more elaborate processes which may be costlier and the extraction process of the metabolites are more difficult and may involve more unit operations.

MATERIAL AND METHODS

Water extraction of bagasse

The bagasse was collected from the local sugarcane juice shop and was cut into small pieces of approximately 1 inch inlength (Figure 1 and 2).A 5 g batchof bagasse was taken in 200ml water, and the water extract was obtained by boiling the bagasse (Figure 3). This water extract was collected at every 5 minutes interval, and continued for up to 60 minutes to facilitate maximum extraction of the processing waste.

Figure 1: Cut pieces of Bagasse,

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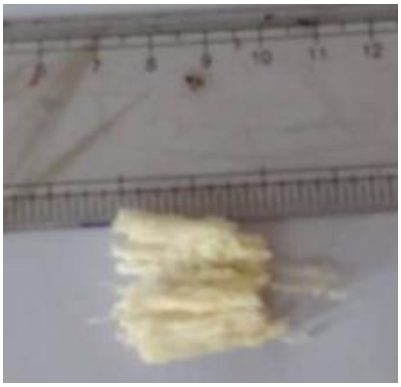

Figure 2: Approximately 1 inch length is cut,

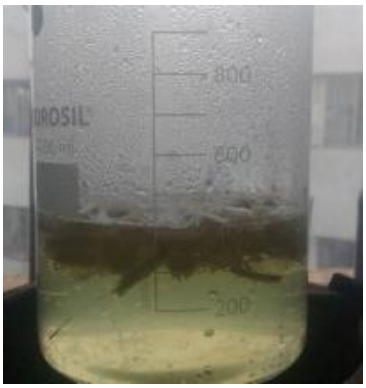

Figure 3: Extraction of bagasse under boiling condition,

Extraction of spent tea

The spent tea leaves were collected from the local tea shops and air dried. 10 g of spent tea was mixed with 150 ml of water,and boiled for 2 to 5 minutes. Five simultaneous extracts were sampled following the same procedure (Figure 4). Each extract was examined singly and then first, second, third, fourth and fifth extracts were respectively added one by one at each step to make a mixed solution.

Figure 4: Spent tea extract up to fifth extraction,

Treatment of potato wash water

The potato wash water was collected from the local potato chips factory. The standard procedure to generate this wastewas estimated from the manufacturer as potato slices soaking in 150 ml water for 15 minutes using (Figure 5). The wash water was taken and subjected to hydrolysis using-5% (v/v) of concentrated

hydrochloric acid under condition of 95° - 100°C for 60 minutes and 1% (v/v)of concentrated hydrochloric acid under condition of 121°C for 15 minutes at 15 psi respectively [17].

Figure 5: Potato wash water from sliced potato (source: Google image),

Estimation of reducing sugar

After completion of the treatment of wastes and neutralization, all the samples were analyzed for estimating reducing sugar by using DNS method (Figure 8) using standard glucose solution[17, 27,28]

Figure 8: DNS method of reducing sugar estimation of samples,

Selection of substrate for fermentation

The estimation of reducing sugaryielded the conditions, under which respective substrate selection would be done for each type of food waste sample. The substrate selection was performed inspecificconditions using fermentation method (Figure 7) by measurement of alcohol. 50 ml of each pretreated sample were taken, and inoculated with 5% (v/v) of inoculum of Saccharomyces cerevisiae (MTCC 180)(Figure 6) strain [24]. The high yielding, 48 hour old culture of S. cerevisiae (MTCC 180) strain was used, growing on Yeast Extract-Peptone-Dextrose media (YEPD)[30]. The temperature was kept at 30-32°C during fermentation, and thereadings were taken at 24, 48 and 72 hrs. After every 24 hours, alcohol content was estimated using dichromate method(Figure 9)[25, 26,29].

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Figure 6: Saccharomyces cerevisiae MTCC 180 strain as streaked slant,

Figure 7: Fermentation of the substrate(s) extracted under anaerobic condition,

Figure 9: Incubation with dichromate for ethanol estimation.

Statistical Analysis

Three replicates of experiments were performed for all the values. Statistical values of standard deviation for each finding were analyzed using one way ANOVA (IBM-SPSS 20) is given in the tables [32].

RESULTS

Reducing sugar of bagasse extract

The readings taken at an interval of every 5 minutes gives the following set of results as shown in Table 1.

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Table 1: Concentration of reducing sugar from bagasse extract at a regular time interval during boiling (95-100° C)

Sample	Concentration(kg/m^3)
Sample 1-5 min	0.5240±0.16^a
Sample 2-10 min	0.533± 0.14^a
Sample 3-15 min	0.576 ±0.23^a
Sample 4-20 min	0.546±0.39^a
Sample 5-25 min	0.551±0.33^a
Sample 6-30 min	0.764±0.19^a
Sample 7-35 min	0.471±0.27^a
Sample 8-40 min	0.523±0.35^a
Sample 9-45 min	0.513±0.42^a
Sample 10-50 min	0.41±0.16^a
Sample 11-55 min	0.523± 0.01^a
Sample 12- 60 min	0.679± 0.11^a

Reducing sugar of spent tea extract

The readings were taken after up to fifth extract and then every time the next extract is added to

Table 2: Concentration of reducing sugar from spent tea extract

Sample	Concentration (kg/m^3)
1st extraction	0.0531±0.02^a
2nd extraction	0.012±0.00^b
3rd extraction	0.0087±0.00^b
4th extraction	0.0087±0.00^b
5th extraction	0.0048±0.00^b
(1st+2nd)extraction	0.028±0.01^b
(1st+2nd+3rd) extraction	0.027±0.01^b
(1st+2nd+3rd+4th) extraction	0.0208±0.00^b
(1st+2nd+3rd+4th+5th) extraction	0.0162±0.00^b

Reducing sugar of potato wash water

The readings were taken after potato wash waterwas subjected to different conditions as shown in Table 3.

Table 3: Concentration of reducing sugar of potato wash water under specific conditions

Sample	Concentration (kg/m^3)
Untreated Potato wash water	0.125±0.02^c
Autoclaved Potato wash water	0.495±0.02^b
Hydrolysed Potato wash water	0.705±0.02^a

Alcohol content for each sample

The most suitable condition for the above three substrate was chosen, and fermentation yielded alcohol concentrations, as shown in Table 4.

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Table 4: Alcohol percentage of food waste substrates at an interval of 24 hours.

Name of the sample	Alcohol content (% w/v)		
	24 hr.	48 hr.	72 hr.
Bagasse extract	0.126±0.00[a]	0.202±0.03[a]	0.094±0.00[a]
Spent tea extract	0.0207±0.00[b]	0.073±0.00[b]	0.047±0.00[c]
Potato wash water	0.054±0.00[c]	0.145±0.00[c]	0.017±0.01[b]

Values in the table are Mean ± S.D of three determinations, superscript letters in a column differ significantly ($p<0.05$)

DISCUSSION

The reducing sugar estimation of the three different waste products and their extracts / hydrolyzed product showed very minor but noticeable difference. When bagasse was extracted for 5 minutes, and continued till 60 minutes, the concentration of reducingsugar remained more or less similar during the initial15 minutesof extractionshowing negligible changes. At 30 minutes, it reached the peakvalue of 0.764mg/ml,after which the reducing sugar content again reduced.Though the statistical analysis shows same significance for all the values; the 30 minutes extraction time was considered in the subsequent experiments, as it gave the highest mean value of all the values.

The water extraction of spent tea clearly showed highest value of 0.0531 mg/ml with the 1st extraction and decreased sharply till 5th extraction. As the extract mixing continued, the value became even lower than the 5th extract and with increase in the mix number it continued to decrease. The significance of the result totally conform to the statistical analysis of the result.

The potato wash waterprepared from sliced potato used for chips processing, yielded a low concentration of reducing sugar, which increased significantly to 0.705mg/ml when the food processing waste washydrolyzed by 5% (v/v) of concentrated hydrochloric acid at 95°-100°C for 60 minutes.it gives almost comparable result with bagasse extract.

After completion of the reducing sugar estimation, the 30 minutes extract of bagasse, 1st extract of spent tea and hydrolyzed potato wash water were used as substrates for fermentation experiments. The estimated alcohol concentration at every time interval showed highest value in case of bagasse extract, with the peak value beingachieved at 48 h.

CONCLUSION

The 30 minutes bagasse extract can be suitably used as fermentation media for the strain Saccharomyces cerevisiae (MTCC 180). However, optimization of fermentation conditions and specific nutritional supplementation(s) must be done to ensure better yield, and food waste utilization potential of the process.

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