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RESEARCH NOTE

Bioethanol fermentation from kitchen waste using *Saccharomyces cerevisiae* [version 1; peer review: 2 not approved]

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Abstract

Bioethanol obtained from microbial fermentation can replace conventional fossil fuels to satisfy energy demand. In this respect, a fermenting isolate of *Saccharomyces cerevisiae*, obtained from date juice, was grown in YEPD medium as a part of a previous published research project. In this study, the isolate was tentatively characterized for alcoholic fermentation in organic kitchen waste medium, prepared from discarded fruit and vegetable peels. Fermentation in shaking condition resulted in the production of 7.3% (v/v) ethanol after 48 h, after which the pH of the medium increased slightly in response. Further research should be conducted to assess the potential of kitchen waste as a raw material in ethanol fermentation.

Keywords

Kitchen waste; *Saccharomyces cerevisiae*; Fermentation; Bioethanol

Open Peer Review

Approval Status

	1	2
version 1
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Introduction

Kitchen waste is a raw material available in large volumes. The term applies to organic solids, which are discarded during food preparation. Kitchen waste is mostly composed of lignocelluloses and starch, and is degradable through microbial infestation. An outstanding resource for biotechnology is present in kitchen waste as carbohydrate polymer fraction¹. The usage of lignocelluloses as feedstock would prompt novel challenges for biotechnology, for example, the product diversification^{2,3}.

Kitchen waste extracted bioethanol is an alluring and sustainable energy source for vehicle fuel, as a gasoline alternative. Present ethanol production (so-called 'first generation'), utilizing harvests such as sugar cane and corn, has become conventional method, whereas second-generation ethanol generation uses less expensive, non-sustenance feedstocks, for example, lignocelluloses or municipal solid waste, which could make ethanol more competitive alternative to petroleum^{4,5}.

Plant cell walls are mostly composed of lignocellulosic biomass. Among its main components is cellulose, a linear polymer of cellobiose consisting of two D-glucose molecules connected by β -1, 4 bonds⁶. The more organized or crystalline cellulose is, the less soluble and less degradable it is. Cellulose degradation techniques include the use of effective enzymes, concentrated acid or alkaline and high temperatures for both nebulous and crystalline cellulose in the transformation procedure. Cellulose would be an ideal carbohydrate source for the fermentation due to the uniform hydrolyzable glucose building blocks⁷.

Hemicellulose is another important hetero-polysaccharide present in the plant cell wall. Hemicellulose differs from cellulose by the organization of several sugar units, by the presence of shorter chains and by a ramified central chain. Hemicellulose removal from the plant cell wall is easier than lignin and cellulose, owing to the bonds between cellulose, hemicellulose and lignin. A wide variety of enzymes, including endoxylanase, exoxylanase, mannanase, arabinosidase, acetylerase, and glucuronisidase, are essential due to its structural diversity⁸. Alkaline pretreatments were also found to be successful at degrading hemicellulose⁹.

Lignin provides additional strength and protection to prevent enzymatic activity of fungi and insect attack by linking cellulose and hemicellulose¹⁰. Substantial moisture and rigidity resistance also added to biomass¹¹ and known as a cellulase inhibitor¹². As a result, exogenous proteins, such as bovine serum albumin, and surfactants, such as MgSO_4 and CaCl_2 ¹³, are added before microbial or enzyme loading. Moreover, many pretreatment processes have been established to moderate the lignin hindrance.

Functional groups of lignin, including phenolic hydroxyl, benzyl hydroxyl, methoxyl, carbonyl and a minor amount of terminal aldehyde groups, are factors that influence its decomposition¹⁴. The lignin carbohydrate complexes (syringyl, guaiacyl and

p-hydroxyphenyl units) formed by crosslink interactions, are also counted as a fermentation-restricting factor.

Starch (α -D-glucose monomer) degradation is complex than the sugar fermentation process. It initially broke down into glucose, through amylase or diastase and maltase hydrolysis. Then, ethanol and carbon dioxide are fermented from sugars through enzyme activity.

Alongside a mainstream project^{15,16}, in this study, tentative fermentation was carried out in kitchen waste medium to find out if a wild-type microorganism has the ability to ferment cellulose efficiently¹⁷.

Methods

Composition and pretreatment

Yeast samples (*Saccharomyces cerevisiae*) were isolated and identified from date-juice using previously described methods^{15,18} in YEPD medium (10^6 – 10^7 cells/ml; 0.3% yeast extract #Y1625, 1% peptone #P7750, 2% dextrose #G8270, 1.5% agar #A1296, pH: 5; Sigma-Aldrich, St Louis, MO, USA). Discarded solid kitchen waste was collected from different households. This included peels from potatoes, pumpkin, papaya, cucumber, okra, green banana, balsan apple, carrot and basil. After chopping, pulverizing and blending with 1 L water, 250 g solid waste was taken as raw medium. Concentrated HCl (2 ml) was added to convert the calcium present (a fermentation inhibitor) to calcium sulfate salt¹⁹. HCl also regulates the pH of the medium to control for bacterial contamination and facilitate chemical hydrolysis of plant residues, which were boiled for 1.5 h, giving carbohydrate units of cellulose and starch. Monomers of amylose, amylopectin and glucose arose from further degradation. Urea (0.1 g) was also supplemented prior to boiling as a nitrogen source nutrient. The final pH was adjusted to 6.0 by dropwise addition of NaOH or HCl (measured using a pH meter; Mettler Toledo, Switzerland).

Fermentation

The 250 ml fermentation medium was transferred into 500 ml Erlenmeyer flasks and a homogenous suspension of yeast (10 ml YEPD broth) was inoculated in aseptic conditions. The flask was incubated in a rotary incubator (120 rpm) at 30°C for 48 h. Two separate experiments were conducted and ethanol production was recorded at 24 and 48 h intervals, and the average were calculated. The ethanol in this experiment was analyzed using the Conway method²⁰. Downstream processing is required before isolation of usable ethanol.

Results

Previous investigations^{15,16,18} indicated that ethanol is produced more readily under shaking than non-shaking conditions. After 48 h of fermentation at room temperature in a rotary incubator (120 rpm), a maximum of 7.3% (v/v) ethanol production was recorded (Table 1). The rate of alcohol production showed a cumulatively increasing trend, which was mirrored by a continued rise in pH throughout incubation, recorded as pH 6.52 at

Table 1. pH change and alcohol production after 48 h fermentation.

Alcohol production (v/v)						
Initial	24 h			48 h		
	1st	2nd	Avg.	1st	2nd	Avg.
0	3.39%	3.34%	3.37%	7.4%	7.1%	7.3%
pH change						
Initial	24 h			48 h		
	1st	2nd	Avg.	1st	2nd	Avg.
6	6.28	6.39	6.34	6.45	6.59	6.52

48 h. The results also indicated that the full potential of kitchen waste fermentation will be revealed through longer durations of fermentation.

Discussion

The kitchen waste medium contained plant organelles and was a rich source of cellulose, starch and glucose monomers. Comparison with previous studies^{15,16,18} showed the achieved production efficiency is below the level required for profitable commercial production. In this study, production of 7.3% (v/v) ethanol was recorded (Table 1). Further optimization of the process and co-fermentation (e.g. ethanol–butanol co-fermentation) is among the future goals of researchers²¹.

Most of the kitchen waste was similar feedstock to lignocellulosic raw materials, which is considered to be an excellent substrate²². Previous works delineated the pathway of converting plant-based waste biomass to bioethanol, in which enzyme pretreatment was conducted before yeast fermentation^{23–26}. Gnansounou & Dauriat produced 30.9 g bioethanol and 65.2 L biogas using 1 kg of kitchen waste²⁷. Velasquez & Ruiz fermented 346.5–388.7 l/ton bioethanol in a similar study using banana pulp and skin²⁸. Industrial waste has also been used in fermentation technology²⁹. A planned facility in East London will produce 16 million gallons of jet fuel per annum from 500,000 tons of waste for British Airways (the Green Sky project). A combination of plasma arc gasification with the Fischer-Tropsch

method, known as Solena's Plasma Gasification (SPG) technology, will be used. In China's Jiangsu province, a *new waste-to-energy facility* with a processing capacity of 900 metric tons, will be constructed.

Conclusions

Results were derived from limited parameters and only a single isolate of microorganism was employed. Future studies should be directed towards elaborate characterization and compare different criteria of kitchen waste fermentation. Optimization of the media and physicochemical parameters, and longer-duration fermentation will also be performed in future. This study was aimed towards fermentation only. A cheap, efficacious downstream processing method of the ethanol generated in this process also requires development.

Data availability

All data underlying the results are available as part of the article and no additional source data are required.

Author information

When the research was carried out, SSR was a MSc student at Biotechnology program, Department of Mathematics and Natural Sciences, BRAC University and NC was the Coordinator of Biotechnology and Microbiology programmes at the Department of Mathematics and Natural Sciences, BRAC University.

Competing interests

No competing interests were disclosed.

Grant information

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The current version of the submitted article doesn't have sufficient content and merit to be indexed as a full journal article. The storyline of the article is not well developed, the relevant references are not well cited, the article doesn't have sufficient results for critical analysis (and how these results are different from other reported results, unless those results are unique and reported for the first time).

It can be included in a conference proceedings (or may not); however, for journal articles we expect high standard research work and publication.

- This article reports the production of bioethanol from kitchen waste fermentation.
- Not technically sound.
- The article does not add any new information to the scientific community.
- Very limited results.
- No comparison/report on similar work around the world.
- This article is not suitable for indexing.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 19 June 2018

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Parveen Fatemeh Rupani

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The manuscript has lack of novelty in term of methodology procedure.

The introduction I suggest to focus more on the previous studies of generating ethanol from kitchen waste and show the gap in between. Clear the objective of the study.

The methodology has not been explained well. The process was under anaerobic or aerobic condition? What was the pH selected for the experiment? No explanation of the pre-treatment of the material, the initial composition characteristics. Also authors did not mention about the size of the sample taken.

Authors have not describe the analytical method used in the study. Full description of the equipment and the methods required.

Authors have to explain in details how many samples were examined, how many runs. And also provide the statistical data in order to discuss the significances of the results.

The results and discussion is very weak and only presented one table. Which is not in scientific

form. Discussion should be with comparison of the previous studies.

Therefore, I do not recommend for indexing.

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Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

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Are all the source data underlying the results available to ensure full reproducibility?

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Are the conclusions drawn adequately supported by the results?

No

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