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PROCESS OPTIMIZATION AND PRODUCTION OF BIOETHANOL FROM VEGETABLE WASTE

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ABSTRACT

Second-generation biofuels, made from sources like wood, straw, microalgae, and vegetable waste, offer an eco-friendly alternative to fossil fuels. The study aims to investigate the fermentation of four types of vegetable waste (carrot, cucumber, potato, and pumpkin) using Saccharomyces cerevisiae to optimize the process for maximum ethanol yield. Optimal conditions for fermentation of carrot, pumpkin and potato were 30°C and pH 4 with maximum ethanol yield of 4.18%; whereas for cucumber, it was 37°C and pH 5. Further improvements, such as pretreatment methods and employing efficient strains of yeast, could enhance the process and enable large-scale implementation, effectively moving towards a zero-waste paradigm.

Keywords: Fermentation, *Saccharomyces cerevisiae*, Specific gravity method, Waste management

Second-generation biofuels, produced from diverse sources such as wood, straw, micro-algae, or biological remains, play a vital role in substituting fossil fuels, leading to a continuous increase in their production. Energy management practices further contribute to ensuring the economic and sustainable production of these crucial biofuels. (Gosavi *et al.,* 2017).

Utilizing fruit and vegetable wastes, which are rich in sugars, as a feedstock for bioethanol production presents an excellent opportunity for cost-effective renewable energy generation. By doing so, not only would the production costs be reduced, but it would also address the pressing challenge of waste disposal effectively (Bhuvaneswari and Sivakumar, 2019).

To achieve a sustainable environment, it is essential to develop household waste management strategies that prioritize eco-friendliness and adopt a biocentric approach, wherein humans play a pivotal role. Biocompost and biogas, along with efficient waste processing measures, play a crucial role in recycling waste and aiming for the ambitious goal of zero waste. One noteworthy best practice is the production of bioethanol through the fermentation of various biomass types. Bioethanol not only serves as an alternative and cost-effective source of fuel but also stands as a renewable energy option. In the realm of bioethanol production, diverse fermentation technologies have been harnessed, utilizing microorganisms like *Saccharomyces cerevisiae* and *Zymomonas mobilis* (Zabed *et al.,* 2017). A co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae* can be used to produce bioethanol from potato waste (Chamchoi, 2019). Furthermore, the substrates used for fermentation exhibit great variability, encompassing agricultural wastes, fruit residues (Kumar and Senan, 2020) and vegetable scraps, as well as pretreated wood (Romani *et al.,* 2010).

An estimated 90-100 million tons of food is wasted each year with the potential to rise beyond 120 million tons by 2020 if direct and immediate action is not taken (FAO, 2015). A substantial portion of the world's greenhouse gas emissions, estimated at 8-10%, is directly attributed to the wastage of food. Minimizing food waste at retail, food service, and household levels holds the potential for diverse benefits, positively impacting both humanity and the environment (UNEP, 2021). By converting food waste into bioethanol, a renewable and cleaner energy, its organic matter is effectively diverted from landfills, reducing environmental pollution and mitigating greenhouse gas emissions.

Within the scope of this study, *Saccharomyces cerevisiae* was employed to ferment four distinct types of vegetable wastes, and through precise experimentation, the optimal conditions for the fermentation process were determined. The objectives of this study are to investigate the potential of using vegetable wastes (carrot, cucumber, potato and pumpkin) as a feedstock for bioethanol production and to optimize the fermentation process for maximum ethanol yield.

MATERIALS AND METHODS

Preparation of the substrate

Four types of household vegetable wastes carrot,

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cucumber, potato and pumpkin procured from Konni market were freshly used for the study (Fig. 1a, 1b, 1c, 1d). The vegetable peels were chopped to 1-2 cm long pieces. 100g of each sample was homogenized with 500ml of distilled water and filtered.

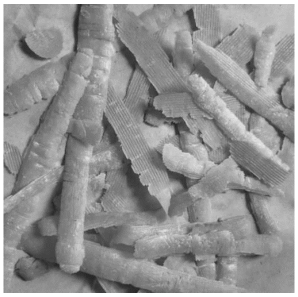

Fig. 1a. Peel of carrot

Fig. 1b. Peel of cucumber

Fig. 1c. Peel of potato

Fig. 1d. Peel of pumpkin

Preparation of inoculum – immobilized yeast

The yeast cells were immobilized in calcium alginate according to the method described by Hermann *et al.* (2021) and Neelakandan and Usharani (2009) with slight modifications. Commercially available instant dry yeast (food grade quality) was used for the study. Five grams of yeast was mixed with 10 ml of lukewarm water, and the resulting slurry mixed with 10 ml 4% (w/v) sodium alginate. This slurry was extruded as discrete droplets using a 1 ml micropipette to obtain beads with a diameter of 0.5 mm, and they were then placed into 100 ml of 0.1M $CaCl_2$ solution. The beads were left in the $CaCl_2$ solution for 30 minutes to set, rinsed twice with sterile deionized water, and stored in test tubes closed with cling film at 4^0C (Fig. 2).

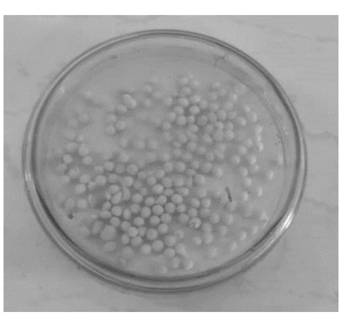

Fig. 2. Immobilized Yeast

Batch fermentation

Fifty milliliters of sterile extract each from carrot, cucumber, potato and pumpkin were transferred into 250 ml conical flasks and sterilized at 121°C, 15lbs for 20 minutes. After cooling, the flasks were inoculated with 25 immobilized beads, plugged with cotton, and kept undisturbed. At 24 hour intervals, 1 ml of sample was withdrawn using a sterile glass pipette for the measurement of specific gravity (SG), percentage of alcohol, and reducing sugar. The amount of reducing sugar was estimated colorimetrically by Dinitro Salicylic acid method (Wood *et al.,* 2012), using 1mg/ml of maltose as the standard.

Determination of the percentage of alcohol (Ethanol) by the SG method

This was performed using the specific gravity method (Nyong *et al.,* 2021). The difference between the SG before and after fermentation is attributed to the conversion of sugars into alcohol during the fermentation process. The procedure involved weighing an empty bottle, filling it with the liquid whose SG needed to be measured, and then reweighing it. The difference in weight was divided by the weight of an equal volume of water to obtain SG of the liquid.

The percentage of alcohol was determined using the following formula.

%v/v alcohol = $(SG_2 - SG_1) / 0.0074$

where -

SG_1 represents the initial SG measurement

SG_2 represents the final SG measurement.

Effect of temperature and pH on the fermentation process

The rate of alcohol production is at its maximum under optimum conditions. To determine the optimum temperature, fermentations were carried out at 25°, 30°, 37° and 40°C. After five days of fermentation, SG and concentration of ethanol (CE) were determined. The rate of alcohol production is at its maximum under optimum conditions. Hence, to determine the optimum pH, fermentation was conducted at pH levels of 4, 5 and 6, while maintaining an optimum temperature of 37°C. After five days of fermentation, SG and CE were determined using a digital pH meter.

At the end of fermentation, a simple distillation was carried out at a temperature of 78°C and bioethanol was collected.

RESULTS AND DISCUSSION

Vegetable waste, a known nonedible source of lipids, amino acids, carbohydrates, and phosphates, can be

Table 1. **Effect of SG on the fermentation of ethanol in carrot, cucumber, potato & pumpkin peel.**

	Carrot peel		Cucumber peel		Potato peel		Pumpkin peel	
	SG (SG1-SG2)	**CE %**	**SG (SG1-SG2)**	**CE %**	**SG (SG1-SG2)**	**CE %**	**SG (SG1-SG2)**	**CE %**
1	0.0235 ± 0.001	3.17 ± 0.09	0.0264 ± 0.002	3.57 ± 0.24	0.0356 ± 0.002	4.81 ± 0.16	0.0338 ± 0.001	4.57 ± 0.07
2	0.021 ± 0.001	2.90 ± 0.14	0.0256 ± 0.001	3.46 ± 0.06	0.0312±0.0002	4.22 ± 0.02	0.0312±0.0003	4.22 ± 0.04
3	0.0152 ± 0.001	2.05 ± 0.08	0.0245 ± 0.002	3.31 ± 0.21	0.0288±0.0003	3.89 ± 0.02	0.0286 ± 0.001	3.86 ± 0.09
4	0.0065 ± 0.001	0.88 ± 0.05	0.0234 ± 0.002	3.16 ± 0.08	0.0256 ± 0.001	3.46 ± 0.12	0.025 ± 0.001	3.38 ± 0.04
5	0.0045 ± 0.001	0.61 ± 0.20	0.0165 ± 0.001	2.23 ± 0.11	0.0245 ± 0.002	3.31 ± 0.20	0.0212±0.0001	2.86 ± 0.01

As fermentation progresses, the SG eventually reaches a constant value, indicating the completion of the fermentation process.

utilized for bioethanol production due to its lignocellulosic composition consisting of 30–50% cellulose, 20–40% hemicellulose, and 10–15% lignin. The hydrolysis method in the presence of microorganisms converts the hemicellulose and cellulose into sugar, with vegetable waste extracts containing around 5% sugar content. (Wilson and Lee, 2014)

Zymase, which is present in yeast, plays a crucial role in converting sugar into ethanol. The study demonstrates that effective fermentation of the substrate leads to the production of ethanol. Additionally, the research determined the optimal conditions required for this fermentation process.

During the fermentation process, the variation in SG (specific gravity) is measured at different stages.

Table 1 presents the effect of SG on CE during fermentation of carrot, cucumber, potato, and pumpkin peel extracts by *Saccharomyces cerevisiae* at 37°C, showing the concentration variation with days of incubation. The maximum ethanol yield obtained for carrot, cucumber, potato and pumpkin peels was 3.17 ± 0.09%, 3.57 ± 0.24%, 4.81 ± 0.16% and 4.57 ±0.07% respectively. These yields were achieved at specific gravity differences (SG1-SG2) of 0.0235, 0.0264, 0.0356 and 0.0338. Beyond these values, there was no significant change in SG, indicating the end of fermentation.

Notably, effective fermentation occurred on the first day itself, and the decrease in the fermentation rate on subsequent days may be attributed to either an increase in CE or a decrease in substrate concentration.

Hossain *et al.* (2010) reported 8.7% ethanol production from rotten pineapple, Kumar *et al.* (2015) achieved 0.15% ethanol using sweet potato, and Kumoro *et al.* (2012) obtained 12.13% ethanol from jackfruit juice as raw material. The highest bioethanol yields were obtained from pineapple waste (0.090%) and sweet potato waste (0.079%), while bioethanol obtained from Indian water chestnut waste and jackfruit waste was comparatively lower at 0.045% (Gosavi *et al.,* 2017). Bioethanol yields for tomato, cabbage and potato were 5.067%, 4.033% and 0.929% (Khandaker *et al.,* 2018). As compared with this study, high yield was obtained for potato extracts.

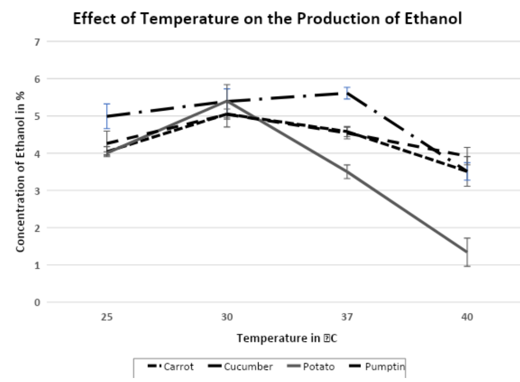

Fig. 3. Effect of temperature on the production of ethanol

Cucumber extracts gave a maximum yield of ethanol of 5.61 ± 0.15 % at 37°C identified as the optimum temperature. [Fig. 3]. This agrees with the findings of (Nyong et al., 2021) who reported 31°C as the optimum temperature for ethanol production from sponge guard, giving a range of 28°C - 32°C. Pretreatment with *Bacillus subtilis*, potato and papaya gave maximum yield under 48-hour fermentation at 30°C (Promon *et al.,* 2018). In co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger* maximum yield was obtained at 30°C on incubation for 96 hours (Subhash *et al.,* 2016).

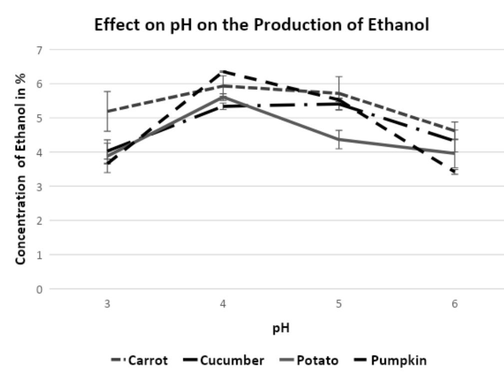

Fig. 4. Effect of pH on the production of ethanol

To study the effect of pH, after a literature study pH of 3, 4, 5 and 6 were chosen. Fermentation performed at respective pH for 5 days at 37°C are represented in Fig. 4. Optimum pH of 4 was noted for carrot, potato and pumpkin while for cucumber maximum yield of 5.40 ± 0.16% was obtained at pH of 5. Studies on fermentation of fruit waste and with yeast showed optimum pH of 4.5 to 6 (Yadav *et al.,* 1997, Babu *et al.,* 2014, Kumar and Senan 2020). The optimal conditions for batch fermentation of yeast are pH 4.25 and a temperature of 30°C (Pramanik 2016).

The fermentation end products were pooled and distilled and the alcohol obtained used for laboratory purposes. Fermentation of wheat straw using bacterial consortium gave a yield of 6.2% of bioethanol (Srivastava *et al.,* 2021). The highest bioethanol production from fruit and vegetable waste using Saccharomyces cerevisiae is achieved at pH 4, temperature 32°C, and a yeast concentration of 3g/L. (Khandaker *et al.,* 2021). Yield of bioethanol vary with source and physical parameters for a particular inoculum.

In conclusion, this study showed that effective fermentation occurred in household vegetable waste. Rise in consumption of natural products pose a threat to the environment. Also, the economic concerns and environmental demands urge the need for alternative sources of energy. Use of household waste in an eco-friendly manner is cost effective. The yield can be increased by chemical or enzymatic pre-treatment methods to remove the lignin before fermentation. Highly efficient or recombinant strain of yeast and saccharification methods if employed can help in proper household waste management and can be standardized on a large scale enabling the concept of zero waste.

Authors' contribution

Conceptualization of research work and designing of experiments (SA, PSV); Execution of field/lab experiments and data collection (TN, ICN, NRS); Analysis of data and interpretation (SA, PSV); Preparation of manuscript (SA, TN).

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