Bioethanol production from rice husk of Dahanala red naadu using *Saccharomyces cerevisiae* and yield enhancement by optimization of growing conditions

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Abstract: The world currently relies predominantly on non-renewable fossil fuels, leading to a severe energy crisis marked by escalating oil prices and detrimental effects of fossil fuel combustion. Over-consumption of these fuels is causing widespread air pollution through greenhouse gas emissions, contributing to hazardous environmental crises such as the greenhouse effect, global warming, and acid rain. This urgent situation underscores the need for a swift transition to sustainable energy sources. Since the paddy plant parts other than grain are underutilized in most of the countries, the usage of paddy husk as a substrate for the bioethanol production was tried. Thus, the objective of the research was to identify the most efficient variety of paddy husk source for bioethanol production in Sri Lanka and to optimize the fermentation conditions in order to improve the ethanol yield. When the husk of different paddy varieties such as Pachapperumal, Sammba, Rath suwandel, Dahanala Red naadu, Dik wee, Kuruluthuda and Naadu rice were used as substrate for the action of *Saccharomyces cerevisiae*, significantly higher amount of bioethanol was produced with Dahanala type of long-grain red rice variety, thus it was selected for further studies. When the substrate concentration of the medium was optimized as 90%, the ethanol yield showed a significant increase by 2.6 times than the control 25%. When the media pH was optimized as 6.0, bioethanol output was increased significantly by 1.2 times than the control pH (7.0). When the incubation period was optimized as 30 hours, significantly higher ethanol yield was obtained. Ethanol production was significantly higher at 25ºC than the control (room temperature). After the optimization, the bio ethanol yield showed a significant increase by 9.4 times in Dahanala type paddy husk than that of the conditions non-optimized. A comprehensive fermentation study, conducted on a large-scale using bioreactors, is imperative to assess the commercial viability of the identified findings.

Keywords: Bioethanol, Dahanala red naadu, Fermentation, Paddy husk, Saccharomyces cerevisiae

1. INTRODUCTION

Principal energy source of the globe at present is still non-renewable petroleum- based fossil fuels such as natural gas, oil and coal which have been actively used for fuel and electricity production (Uihlein & Schbek, 2009). Current rate of combustion of the fossil fuels would lead to the worldwide environmental crisis. The increase in demand of fossil fuels and depletion of natural fuel reserves have led to the development of eco-friendly substances (Du *et al*., 2008; Pande & Bhaskarwar, 2012). Industrialization and human population are showing an increasing trend and these demand higher level of energy production. Biomass is one of the important alternative energy sources to fossil fuels (Tatli, 2009; Balat, 2010; Darici and Ocal, 2010).Most popular renewable fuel in the transportation sector is ethanol (Carere *et al*., 2008). Global ethanol production has been showing a healthy increase since the beginning of oil crises in early 1970 (Asmamaw & Fassil, 2014).

Bioethanol production using crop and non-crop raw materials are recent developments. Diverse agro wastes could be used as sources for the production of biofuel since they contain a significant amount of polysaccharides. However, continuous usage of food crops for biofuel production has been widely criticized due to the higher demand of food and feed for increasing population (Tatli, 2009; Balat, 2010; Darici & Ocal, 2010), rising food prices and large scale deforestation for the purpose of cultivation of energy crops. Hence, there is a serious demand to develop more sustainable alternatives that do not impact the worldwide

production of food (Widyaningrum *et al*., 2016). Rice is a staple food that plays as the traditional diet of a huge percentage of human population. Worldwide production of paddy husk is very significant and the average range of paddy falls around tens of millions of tons per annum. Paddy plant parts have high potential for biofuel production since they can be grown under different environmental conditions all over the Asia and Africa and have the capacity to produce bioethanol continuously under the provided conditions. Large proportion of paddy husk is either dumped as waste or used as substrates or nutritive source for the soil. Generally, large amounts of rice husk and paddy plant left over are dumped as wastes that results in issues in the waste disposal system and emissions of methane gas (Irfan *et al*., 2014). The remainder is sold to other industries for fertilizer production (Sookkumnerd *et al*., 2005).

Bioconversion of paddy husk to a useful form of energy will meet the energy requirement of the mills. This will minimize the issue of waste disposal in addition to converting rice husk to a renewable energy resource (Saha *et al*., 2005). For these reasons, research and development is underway for the production of bioethanol from the paddy husk, bran, dried paddy leaves that remains after the removal of paddy grains. However some unused parts of paddy have rigid structures and contain components that cannot be easily converted into ethanol (Irfan *et al*., 2011, 2014).

Since Sri Lanka has a developing economy based on the paddy cultivation mainly and rice is the staple food and the crop occupys nearly 30% of the total agricultural lands, large amount of paddy husk left after the cultivation can be utilized (Rodrigo & perera, 2011). In Sri Lanka, there are different rice varieties used for cultivation in different parts of the country. In this study, rice husk was chosen because it consists of cellulose and lignocellulosic substances and the sugar monomers that could be fermented to produce ethanol and found to be an appropriate alternative energy source. Further, getting the husk of any variety of rice has been less expensive and rice grows excessively all over Sri Lanka. Thus, the objective of the research was to identify the most efficient variety of paddy husk source for bioethanol production in Sri Lanka and to optimize the fermentation conditions in order to improve the ethanol yield.

2. METHODOLOGY

2.1 Collection of raw materials

Saccharomyces cerevisiae, commonly known as baker's yeast, was purchased from Cargills Food City and cultured on a peptone, yeast extract, and nutrient (PYN) medium. (Christy *et al*., 2021). Paddy husks of different rice varieties such as Pachapperumal, Sammba, Dahanala red naadu, Rath suwandel, Dik wee, Kuruluthuda and Naadu rice were collected from the different paddy fields located in different parts of the western Province of Sri Lanka.

2.2 Chemicals and Media

All chemicals employed were sourced from standard biochemical suppliers, ensuring quality and reliability (Christy *et al*., 2023a).

2.3 Bioethanol production and measurement

In the fermentation medium (100 mL), an inoculum of 5 g/L was introduced and then incubated at 25°C in a rotatory shaker set to 150 rpm. Each flask was cultured under oxygen-limited conditions for 24 hours, achieved by sealing it with parafilm and storing it in an anaerobic chamber. After centrifugation, the supernatant was used to measure the bioethanol content.

2.4 Optimization of culture conditions for bioethanol production

2.4.1 Effect of different type of paddy husk as substrate

Different paddy varieties such as Pachapperumal, Sammba, Rath suwandel, Dahanala Red naadu, Dik wee, Kuruluthuda and Naadu rice were used as substrate with the liquid fermentation media.

2.4.2 Effect of substrate concentration

The fermentation media were prepared using varying concentrations of Dahanala Red naadu rice husk substrate (5% - 100%) in the liquid fermentation medium. Bioethanol production was done and measured as per section 2.3.

2.4.3 Effect of pH

The fermentation media were prepared by making Dahanala Red naadu rice husk at 90% concentration with liquid fermentation media at various pH values (3.0 - 10.0 pH). Bioethanol production was done and measured as per section 2.3.

2.4.4 Effect of incubation period

Ninety percent concentration of Dahanala Red Naadu rice husk was filled into various conical flasks at a pH of 6.0. *Saccharomyces cerevisiae* was introduced into each flask for fermentation. Subsequently, all containers were subjected to incubation with *Saccharomyces cerevisiae.* Every 3 hours, each sample was taken and alcohol activity was measured as per section 2.3. Time used for incubation period were 3, 6, 12, 15, 18, 21, 24, 30, 36, 42 & 48h. Bioethanol production was determined after each period of incubation.

2.4.5 Effect of temperature

Ninety percentage concentration of Dahanala Red naadu rice husk was taken in 6.0 pH and incubated for 30 hours at different temperatures such as 10, 15, 20, 25, 30, 35 & 40^{o}C. Bioethanol production was determined as per section 2.3.

2.5 Statistical analysis

All the experiments were done in triplicates. Statistical analyses were performed using Minitab 17.0 version. Obtained data were analyzed using one way ANOVA. To determine significant difference at p _< 0.05, Tukey's multiple comparision test was performed (Christy *et al*., 2023b).

3. RESULTS

3.1 Effect of different type of paddy husk as substrate

Among the different type of paddy husk, Dahanala Red naadu, was produced significantly higher amount of alcohol than other substrates tested (Figure 1). Therefore, it was selected as best substrate for bioethanol production further studies.

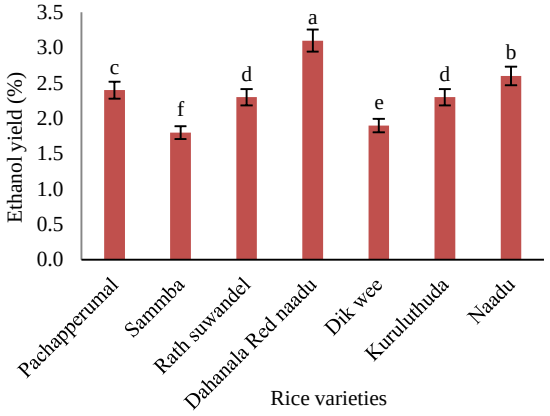

Figure 1: Effect of different rice varieties on bioethanol production using *Saccharomyces cerevisiae*. Values indicated by different letters (a-f) are significantly different (p< 0.05).

3.2 Effect of substrate concentration

When Dahanala red naadu rice substrate concentration was increased from 5 to 100%, a significantly higher amount of ethanol production was obtained at 90% of substrate concentration. At this optimized substrate concentration, the bioethanol yield increased by 2.6 times compared to the non-optimized substrate concentration (Figure 2). Initial sugar content is an important factor in the fermentation process, and it has a direct effect on the fermentation rate and microbial cells. An increase in sugar concentration up to a certain point caused a prominent rise in the fermentation rate (Zabed *et al*., 2014). Further increments in sugar concentration resulted in a steady fermentation rate because the concentration of sugar used was beyond the threshold uptake

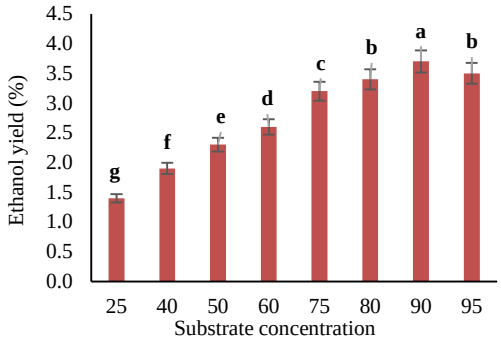

Figure 2: Effect of different substrate concentrations of Dahanala Red naadu on bioethanol production using *Saccharomyces cerevisiae*. Values indicated by different letters (a-g) are significantly different (p< 0.05).

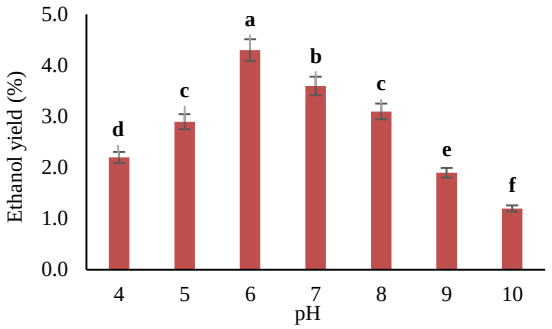

Figure 3: Effect of different pH on bioethanol production from Dahanala Red naadu using *Saccharomyces cerevisiae*. Values indicated by different letters (a-f) are significantly different (p< 0.05).

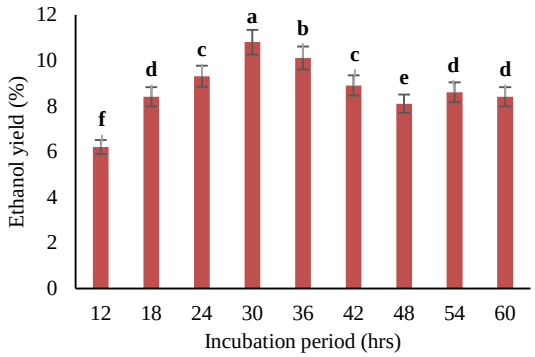

Figure 4: Effect of different incubation periods on bioethanol production from Dahanala Red naadu using *Saccharomyces cerevisiae*. Values indicated by different letters (a-f) are significantly different (p< 0.05).

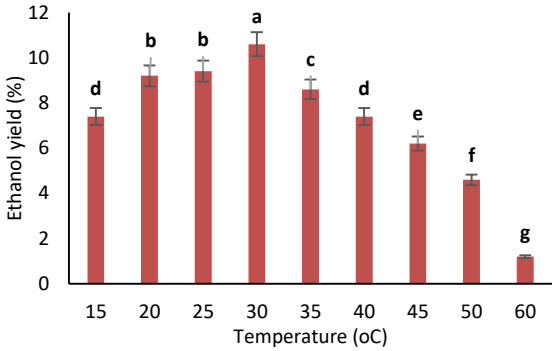

Figure 5: Effect of different temperatures on bioethanol production from Dahanala Red naadu using *Saccharomyces cerevisiae*. Values indicated by different letters (a-g) are significantly different (p< 0.05).

level of the microbial cells (Laopaiboon *et al*., 2007). Higher ethanol productivity and yield in fermentation can be achieved with a higher initial sugar concentration. However, this also results in longer fermentation times and subsequently, increased recovery costs (Laopaiboon *et al*., 2007). Therefore, 90% Dahanala type of long-grain red rice substrate concentration was chosen for further culture growing studies.

3.3 Effect of pH

When the pH of the medium was optimized as 6.0, bioethanol yield was increased significantly by 1.2 times than the non-optimized control pH (7.0 - Figure 3). The pH of the broth significantly affects ethanol production as it directly impacts the organisms involved and their cellular biochemical processes. The permeability of some important nutrients into the cells is impacted by H+ concentrations in the fermentation broth (Piršelová *et al*., 1993; Kasemets *et al*., 2007). Highly acidic fermentation medium will increase the fermentation rate. This is attributed to the increased activity of yeast-produced enzymes specialized for fermenting glucose, which are particularly adept and active in acidic environments. Among these tested pH values, pH 6.0 was selected as the best for bioethanol production with Dahanala Red naadu rice substrate. Hence pH 6.0 was chosen as optimized pH, for further studies.

3.4 Effect of incubation period

When the incubation period of the inoculated medium was optimized as 30 hours, significantly higher ethanol yield was obtained and this is 1.56 times than the non- optimized control. The bioethanol produced after 18 hours, 24 hours, 30hours, 36 hours, 42 hours and 48 hours were 8.4%, 9.3%, 10.8%, 10.1%, 8.9% and 8,1% respectively (Figure 4). The duration of incubation significantly influences microorganism growth. Shorter fermentation periods often result in inefficient fermentation due to insufficient microorganism growth. Prolonged fermentation periods might lead to accumulation of toxic substances that affect microbial growth especially in batch mode due to the higher concentration of ethanol accumulation in the fermented broth (Christy and Kapilan, 2023; Asmamaw & Fassil, 2014). Therefore, 30 hours of incubation period was chosen for further culture growing studies.

3.6 Effect of temperature

When the culture growing temperature of the inoculated medium was optimized as 25^{o}C, significantly higher ethanol yield was obtained and this is 1.8 times than the non- optimized control (30^{o}C – Figure 5). Temperature is directly affects the growth rate of the microorganisms. High temperature will stress the microorganisms and unfavorable for their growth (Kapilan *et al*., 2015). Some microorganisms have the ability to generate heat-shock proteins when subjected to high temperatures, resulting in the deactivation of their ribosomes (Christy *et al*., 2023c; Kapilan & Arasaratnam, 2010). Fermentation process and microbial activity which are regulated by variouus enzymes which are sensitive to higher temperatures since higher degree temperatures distrupt their tertiary structure by inactivating them (Christy *et al*., 2023a; Phisalaphong *et al*., 2006). Microorganisms which facilitate fermentation, have optimum temperature range for showing better growth (Christy *et al*., 2023a, Christy *et al*., 2023d, Ilangarathna, & Kapilan, 2022). Therefore, it is necessary to predetermine an optimal temperature range for fermentation to promote favorable microbial growth and maximize ethanol production. Typically, the optimal temperature range for fermentation is 20–35 °C, and a high temperature during the fermentation can lead to uncontrollable issues (Ballesteros *et al*., 2004). Hence the ethanol production was significantly higher at 25^{o}C than the other temperatures, it was selected as the optimum temperature for the biofuel production under the conditions examined.

4. CONCLUSION

Husk of Dahanala type of long-grain red rice is an effective substrate for bioethanol production. After the optimization of substrate concentration (90%), pH of the medium (pH 6.0), incubation period (30 hours) and the temperature (30^{o}C), the production of bio ethanol was significantly increased in rice husk (12.4 times) than the conditions that were not optimized.

5. ACKNOWLEDGEMENT

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