IJMS 2021 8(3): 101 - 124

International Journal of Multidisciplinary Studies (IJMS)
Volume 8, Issue III, 2021

Utilization of bioethanol generated from papaw peel waste for hand sanitizer production

Amanullah A* and Kapilan R

Department of Botany, University of Jaffna, Jaffna, Sri Lanka

ABSTRACT

Hands are the primary mode for the spread of microbes. For infection control, the first-line of defense as well as personal hygiene, are mandatory. Hand sanitizers that contain ethanol as the main constituent are used to kill a broad range of microbes. Demand for petroleum-derived ethanol is increasing with the COVID-19 outbreak and primary suppliers are searching for alternatives to overcome this problem. Objective of this study is to produce bioethanol from ripen papaw peel waste using Saccharomyces cerevisiae and to determine the potential utilization of bioethanol generated from papaw peel waste for a pilot study of which the end aim is hand sanitizer production. The blended ripened papaw (Carica papaya) fruit peel (100g/L) was inoculated with the S. cerevisiae (2g/L) in a fermentation medium that contains 10 g/L yeast extract, 10 g/L KH2PO4, 2 g/L (NH4)2SO4 and 0.5 g/L MgSO4•7H2O and allowed to ferment for 6-36 hours at room temperature. The bioethanol yield obtained after 12 hours, was 0.6% (V/V). The fermentation conditions were optimized by changing one factor at a time, while keeping the other variables constant. Significantly higher bioethanol yield (6.2 times, 3.7% V/V [$p<0.05$]) was obtained from papaya peels at the optimized conditions of 12 hours of incubation period, 5:1 ratio between air space and fermentation solution, 5g/L of yeast inoculum, 15g/100ml of papaw fruit peel, 1g/100ml of soybean powder as nitrogen source, 60ml/100ml of diluted sulfuric acid at pH 5. When the agar well diffusion assay was performed against pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, Bacillus spp. and Pseudomonas spp, all the bacterial strains showed an inhibition zone, i.e., they were sensitive for the bioethanol extract.

KEYWORDS: *Saccharomyces cerevisiae, Bioethanol, Papaw peel waste, Soybean*

Corresponding author: A. Amanullah, Email: aznaamanullah96@gmail.com

1 INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a respiratory illness that is caused by a novel virus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This disease was first identified in the Hubei province of the People's Republic of China (Zoumpourlis et al. 2020). World Health Organization (WHO) has declared this as a global pandemic and the total number of cases has passed 180 million globally, with 4 million deaths. For infection control, the first-line of defense and personal hygiene are mandatory (Hans et al. 2021). People are always advised to maintain social distancing, wear face masks, clean their hands with sanitizer or soap, avoid crowds, and cough onto a bent elbow. Cleaning hands with sanitizer or soap is a first-line defense (Singh, et al. 2020).

1.1 Hand sanitizer

Hand sanitizer is used to kill a broad range of micro-organisms and they are found in gel, liquid, and foam forms (Jing. et al 2020). Sanitizers are of two types: alcohol-based sanitizer and non-alcohol-based sanitizer (Hans. et al 2021). World Health Organization recommends alcohol-based hand sanitizer since ethanol can kill a broad range of micro-organisms including bacteria, fungus, viruses, and protozoans (Jing. et al 2020). Alcohol-based hand sanitizers contain 60-98% purity ethanol or isopropyl alcohol. Ethanol is non-toxic and less expensive. Therefore, ethanol is preferred over isopropyl alcohol (USFDA, 2020). The annual growth rate of hand sanitizer production has increased from 5.06% (2019) to 45.71% (2020), globally (Fortune Business Insights 2021). Due to the COVID-19 outbreak, hand sanitizer market is likely to improve in upcoming years. Demand for personal hygiene products owing to COVID-19 such as soap, hand wash, hand sanitizer, and tissue are increasing around the globe (Berardi et al. 2020).

1.2 Ethanol

Ethanol or ethyl alcohol is a liquid with a colourless and clear appearance. It is also volatile. Ethanol is produced through biochemical (via fermentation), thermochemical (via gasification), and petroleum-based ethanol production (Bušić et al. 2018). The primary ethanol production is petroleum-derived production by the hydrolysis of ethylene. Synthetic ethanol is produced from natural gas, coal, and ethylene. Petroleum-derived ethanol production is a simple process but this process depends on the primary suppliers of petroleum-based products (Tamers 2006).

Ethanol is the most common alcohol that is found in alcohol-based sanitizers and effective against several viruses (Golin, et al. 2020). Within a short period of time ethanol can kill a broad range of microorganisms. Studies have proven that alcohol-based sanitizers are used both in the interior and in the exterior of healthcare facilities as they effectively kill microbes (Gold, et al. 2021).

Two million tons of petroleum-derived ethanol is produced annually, and the main suppliers are Saudi Arabia and South Africa (Tamers 2006). Due to the COVID-19 outbreak, demand for crude oil is

increasing dramatically, which leads to the demand for petroleum-derived ethanol production. This increases the prices of petroleum-derived hand sanitizer production (Hans et al. 2021). Crude oil is a non-renewable energy source and primary suppliers are searching for an alternative to overcome this concern (Vikramaditya et al. 2020).

Another major source of ethanol production is from fermentation of lignocellulosic agricultural leftovers using micro-organisms and the resulting product is known as bioethanol. Bioethanol is similar to petroleum derive ethanol and it is used as an alternative for fossil fuels, as an ingredient in cosmetics and beauty products, pharmaceuticals, food, and beverages (Vohra et al. 2013). There are four generations of bioethanol namely first generation, second generation, third generation, and fourth-generation (Aron et al. 2020). Bioethanol is produced from simple sugars especially from glucose by fermentation using micro-organisms. Therefore, bioethanol is a renewable source (Vohra et al. 2013). First-generation bioethanol is derived directly from food crops like sugar cane, corn, sugar beet, and soybean. These yield a higher amount of bioethanol at the end. The major countries that produce bioethanol are United States (Corn), Brazil (Sugarcane), and Europe (sugar beets) (Singh, et al. 2015). As first-generation bioethanol is obtained directly from a food source it is strictly prohibited in most countries (Hans et al. 2021). The reason for this mainly depends on food security as more than 768 million people are still starving without food (Food and Agriculture Organization of United Nations, 2020). Second-generation bioethanol mainly relies on non- edible lignocellulosic biomass which considers as a leftover in agricultural activities. The advantage is they are not food crops such as agriculture leftovers, forestry wastes, and organic leftovers (Aron et al. 2020). Such efforts require advanced technologies to hydrolyze cellulose, hemicellulose, and lignin (Goh & Lee. 2010). Third-generation bioethanol is produced with macro algae or cyanobacteria. Fourth-generation bioethanol production is from genetically-modified organisms. Higher cost of production is required for both third and fourth generation bioethanol production (Sikarwar et al. 2017). Considering the sources of the ingredient, cost of production, food security and technologies needed for the production second-generation bioethanol is preferred over other generations of bioethanol. Fruit wastes are considered as lignocellulosic biomasses and they are a good source of second-generation bioethanol since they contain adequate amounts of sugars (Jahid et al. 2018). There are numerous amounts of micro-organisms that can be used for the fermentation process. Among those, *Saccharomyces cerevisiae* has been the best choice for alcoholic fermentation because of the following reasons: efficient capacity to convert sugar into alcohol, the capability of producing a loosely clumped mass of fine particles during growth, easier to settle or suspend in the fermentation chamber (Kosaric et al. 1995) and higher tolerance to the ethanol present in the growing media (Olsson et al. 1993).

1.3 Papaw fruit peel as a source

Ripened papaw fruit peel *(Carica papaya)* is used in this study as they possess lower amounts of lignin content. The lignin content of the biomass directly influences bioethanol production as they cannot be hydrolyzed easily. Papaw fruit peel contains 37.49 g/100g of carbohydrates. This carbohydrate includes 4.15g/100g of sugar and 12.16g/100g of crude fiber (Romella, et al. 2016). Hydrolysis of complex carbohydrates is divided into 3 types. They are acid hydrolysis, base hydrolysis and enzymatic hydrolysis. Sugars and the crude fibers that are found in papaw fruit peel can be converted into simple sugars by acid hydrolysis. Method using membrane filtration of sugar juice is highly preferred over the conventional liming-carbonation method for yielding higher sucrose concentration (Hakimzadeh et al. 2006; Kawa-Rygielska et al. 2013; Regiec et al. 2004; Shahidi et al. 2006). Further, the papaw fruit is very cheap, available, and grows excessively all over Sri Lanka.. Ethanol is the key ingredient of hand sanitizer production. The production of ethanol in a cost-effective way of using papaw fruit peel waste and can be termed as a good trend for the production of hand sanitizers worldwide. The objectives of the research study were to produce bioethanol from papaw fruit peel waste using Saccharomyces cerevisiae and to determine the potential utilization of bioethanol generated from papaw peel waste for hand sanitizer production.

2 MATERIALS AND METHODS

2.1 Source of strain and fruit

Baker's yeast (*Saccharomyces cerevisiae)* was purchased from the local market. Ripened and over ripened (dark yellow to orange colour) papaw (*Carica papaya*) fruits peels were collected from the local market and papaw peel juice were prepared.

2.2 Chemicals and Media

All the chemicals used were obtained from standard sources. Basal medium containing 10 g/L yeast extract, 10 g/L KH2PO4, 2 g/L (NH4)2SO4, and 0.5 g/L MgSO4•7H2O was prepared. For the acid hydrolysis 0.5M sulfuric acid was added to the substrate and it was autoclaved at 121˚C for 30 minutes. After the autoclaving of the conical flask containing 100ml of basal media and hydrolyzed substrate, the chamber was inoculated with 1.0 grams of *Saccharomyces cerevisiae* (10g/L) (Kaewkrajay, et al. 2014).

2.3 Production of bioethanol and measurement

Acid hydrolysis was carried out by treating 10.0 g of ripen papaw fruit peel juice with 40.0ml of 0.5M sulfuric acid at 121^{o} C for 20 minutes in an autoclave. After the acid hydrolysis with heat treatment inoculum (10g/L) was added to the fermentation medium (100mL) and it was incubated at room temperature (30^{o}C) in a rotatory shaker (100rpm). Each flask was cultured at room temperature under oxygen-limited conditions up to 36 hours. The oxygen-limited condition was provided by sealing the flask tightly with parafilm and keeping it in an anaerobic chamber. Resulting suspension was taken and the extract was centrifuged. The supernatant was used for bioethanol measurement.

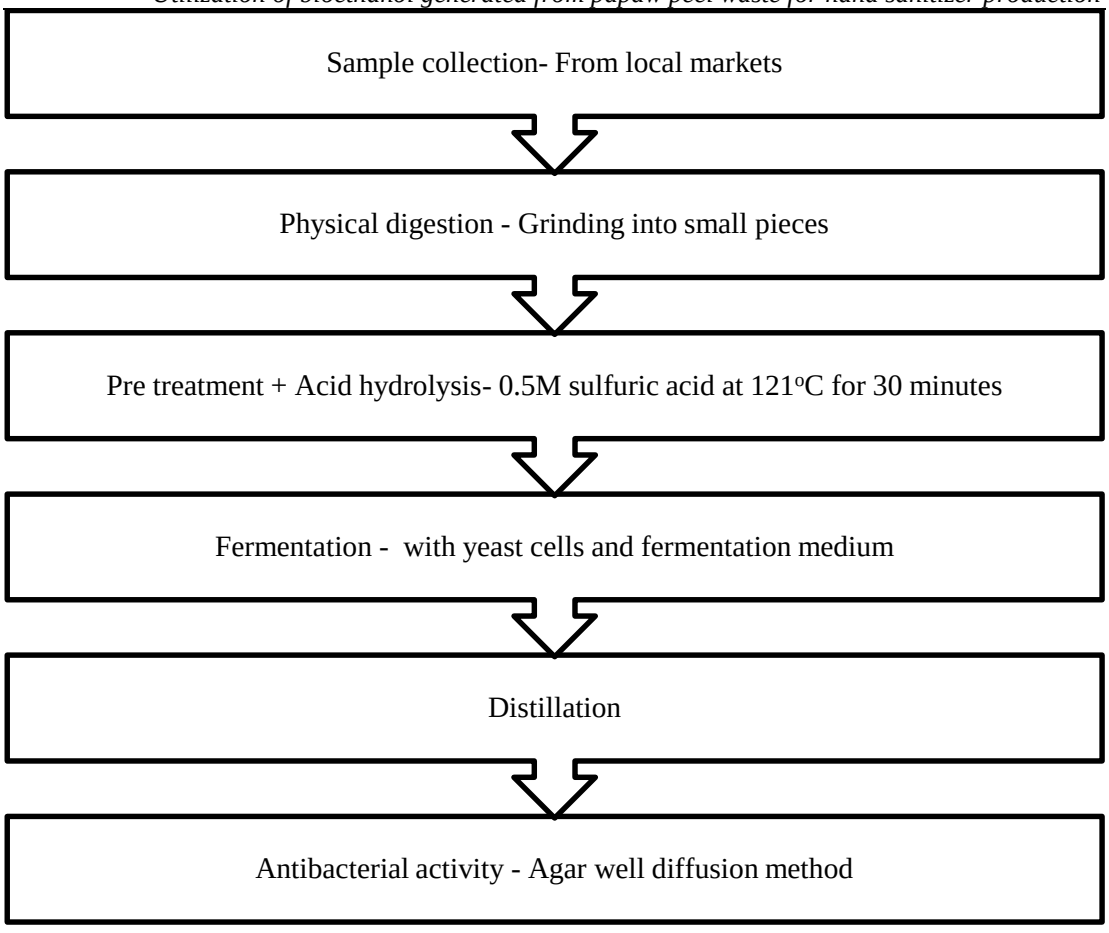

Figure 01: Steps involved in ethanol production

2.4 Determination of sugar content

Sugar concentration was measured by using Dinitro salicylic acid method (Miller, 1959) before and after the fermentation process. The suspension was mixed and the extract was centrifuged for 20min at 3000rpm in a bench centrifuge.

2.5 Bioethanol measurement

The supernatant was used for bioethanol measurement in percentage using ebulliometer (Wahab et al., 2005). To determine the boiling point of water, the lamp was filled with 95% Reagent Alcohol. The boiler was rinsed and poured through the opening, 50.0mL pure water was measured with the sample vial was filled up to the mark. The thermometer was placed in position by inserting into proper opening. The alcohol burner was lighted and placed. Soon after applying heat, the thermometer will register movement and steam will come out of the top vent. When the thermometer reading turned stable, the temperature was read. Example: The reading is 100 degrees and three tenth = 100.3 degrees, the calculating dial was taken and move the circular sliding part until the division 100.3 degrees is directly opposite the zero of the fixed graduation. This is the temperature reading for water to

be used for this set of calculations. The ebulliometer body was moved carefully away from the burner. The thermometer was removed and was set aside on a soft surface. The stopcock was used to drain the water and rinsed carefully. The stopcock was opened, and the boiler was emptied, it was rinsed with some sample to be tested, that was poured out again and it was blew through upper tube to clear away the condensed steam. 50.0mL sample was poured into the boiler by using the sample measure, and was filled up to the particular mark. The thermometer was placed in proper opening, the condenser was filled with cold water, and heat as previously discussed. The mercury was raised and stabilized; and until the mercury is motionless the reading was not taken. For example- the thermometer was read as 99.7, and the comparison of this figure on the scale, read 0.6 degrees. This means that the tested sample has 0.6% of alcohol by volume.

2.6 Optimization of conditions for bioethanol production

2.6.1 Production of bioethanol in papaw peel medium

Fermentation medium (100mL) was inoculated with *Saccharomyces cerevisiae* (1.0g) and incubated at 30^{o}C for 24h. Ethanol production was monitored.

2.6.2 Effect of incubation period

Media were prepared by mixing the substances at the appropriate level in the liquid fermentation media. The medium was set at pH 6.0 and inoculated with yeast inoculums (1.0 grams/100ml) and incubated at 30^{o}C at 100rpm. The set-ups were incubated at incubation periods of 6h, 12h, 18h, 24h, 30h & 36h.

2.6.3 Effect of volume of sulfuric acid

Media were prepared by mixing substances at the appropriate level in the liquid fermentation media. Different volumes of 0.5M sulfuric acid (10ml, 20ml, 30ml, 40ml, 50ml, 60ml, 70ml and 80ml) were added to the 10.0g of substrate and the acid hydrolysis was carried out with heat treatment for 30 minutes. The medium was inoculated with yeast inoculum (1.0 grams/100ml) and incubated at 30^{o}C at 100rpm for 12 hours.

2.6.4 Effect of nitrogen source

Fermentation media were prepared by taking different nitrogen sources (Soybean flour, mung beans flour, corn flour and peptone) in a concentration of 1.5 grams/100mL. The experiment was continued and ethanol production was measured using ebulliometer method.

2.6.5 Effect of amount of nitrogen source (soybean flour)

Acid hydrolysis was carried out. Media were prepared by mixing all the substances with different amounts of soybean powder (0.5, 1.0, 1.5, 2.0 and 2.5 gram/100mL) with 10.0g of papaw peel mixture in liquid fermentation media. The mixture was inoculated with yeast inoculum (1.0 grams/100ml) and incubated at 30^{o}C at 100rpm for 12 hours.

2.6.6 Effect of ratio of the fermenting solution: air space

Acid hydrolysis was carried out. Media were prepared by mixing substances at the appropriate level in the liquid fermentation media. The medium was set at different ratio values such as 1:10, 1:05, 3:10, 2:05 and 1:05 with the fermenting solution. It was inoculated with yeast inoculum (1.0 grams/100ml) and incubated at 30^{o}C at 100rpm for 12 hours.

2.6.7 Effect of inoculum size

Acid hydrolysis was carried out. Media were prepared by mixing the substances at the appropriate level in the liquid fermentation media. Different amount of yeast inoculum (2.5, 5.0, 7.5, 10.0, 12.5 gram/ 100mL) was added in the media and incubated at room temperature (30^{o}C) at 100rpm for 12 hours.

2.6.8 Effect of substrate concentration

Acid hydrolysis was carried out. Fermentation media were prepared by mixing all the substances present in fermentation media in appropriate amount. Different amount of substrate (5g, 10g, 15g, 20g and 25g) was added to the liquid fermentation media. The fermentation medium was inoculated with yeast inoculum (1.5 grams/100ml) and incubated at 30^{o}C at 100rpm.

2.6.9 Effect of pH of the medium

Acid hydrolysis was carried out. Media were prepared by mixing already optimized substances at the appropriate level in the liquid fermentation media. The medium was set at different pH values namely 4.0, 5.0, 6.0, 7.0 and 8.0 and inoculated with yeast inoculum (1.5 grams/100ml) and incubated at 30^{o}C at 100rpm for 12 hours.

2.7 Distillation of end product

After the optimization the resulting end product was distilled using distillation assembly for 4-6 hours (Chitranshi and Kapoor, 2021).

2.8 Antibacterial activity

Agar well diffusion method was used to determine the antibacterial activities of distilled bioethanol extract which was produced from papaw fruit peel mixture. 4 strains of human pathogenic bacteria were used for this study. They were *Escherichia coli, Staphylococcus aureus, Bacillus spp. and Pseudomonas spp.* Nutrient agar plates were made by pouring appropriate amount of nutrient agar into sterilized petri dishes. 1.0 ml of fresh bacterial culture was pipetted into the center of a sterile Petri dish and spread using a spreader. Wells with 6.0mm diameter were made using a sterile cork borer in the nutrient agar plates. Then, 100 µl of bioethanol extract was added to wells and the plates were incubated at 37°C for 24 h. Antibacterial activity was detected by measuring the zone of inhibition that appeared after the incubation period (Omar et al. 2013).

2.9 Statistical analysis

All the experiments were made in triplicates and the mean values were used to plot the graphical representation.

Statistical analyses were performed using Minitab 18.1 Version. The data were analyzed using one way ANOVA. Tukey's multiple comparison tests were used to determine significant differences at $p < 0.05$.

3 RESULTS & DISCUSSION

3.1 Production of bioethanol in papaw fruit peel medium

The amount of ethanol produced from the papaw peel mixture was 0.6% under non-optimized conditions after 24 hours of fermentation. Sugar concentrations before the commencement of the experiment and after optimization of the fermentation process, was measured by using 3, 5-Dinitro salicylic acid method and tabulated in Table 01.

The process of ethanol production is a two-step process. Acid-hydrolysis converts cellulose to glucose sugars by hydrolysis (saccharification) and the resulting sugars can be converted to ethanol by fermentation. Papaw fruit peel contains a larger amount of crude fibers that can be converted into simple sugars for fermentation. The purpose of acid hydrolysis is to convert polysaccharides (cellulose and hemicellulose) and disaccharides (sucrose and maltose) into simple sugars (glucose) for the fermentation, in order to produce bioethanol (Iranmahboob, et al. 2002).

Table 01: Sugar concentration measurements before & after the fermentation by using 3, 5-Dinitro salicylic acid method (Miller., 1959)

	Before the acid hydrolysis	**Before the fermentation (After hydrolysis)**	**After the optimization of fermentation conditions**
3,5-Dinitro salicylic acid method(at 540nm)	0.24 $moldm^{-3}$	0.42 $moldm^{-3}$	0.17 $moldm^{-3}$

3.2 Effect of the incubation period

The bioethanol production after 6h, 12h, 18h, 24h, 30h and 36h of fermentation by yeast were 0.36%, 0.80%, 0.70%, 0.60%, 0.28%, and 0% respectively (Figure 01). There was a significant difference with 12h of fermentation period in relation to alcohol yield, and consequently, it was decided to experimentally use 12h as the incubation period for future experiments. Short fermentation time causes inadequate growth of microorganisms within the fermentation medium that results in inefficient fermentation. Long fermentation time causes an inhibitory impact on microorganism's growth due to the presence of a higher concentration of ethanol in the fermented broth. (Asmamaw et al. 2014; Hossain et al. 2011; Nadir et al. 2009). High level of alcohol concentration leads to inhibition of ethanol production by inactivating the growth of *S. cerevisiae* in the media (Zabed, et al. 2014). Total sugars present in the medium decreases with time due to the consumption of sugars by yeast cells. Furthermore, ethanol yield is decreased due to incomplete substrate consumption by yeast cells (Hosny, et al.

2016). Production of ethanol in a continuous process by S,.cerevisiae reduced with a longer cultivation time (Azhar, et al. 2017).

3.3 Effect of acid on hydrolysis

When the volume of the acid is 60ml/100ml, the ethanol yield was significantly increased by 1.09 times (2.30% to 2.50%). Control setup without acid hydrolysis shows significantly lower amount (0.17%) of ethanol production when comparing with hydrolyzed substrates (Figure 02). This shows that polysaccharides and disaccharides were converted into simple sugars through acid hydrolysis that was used for fermentation process. Sulfate is a nutrient present in the fermentation medium. Therefore, sulfuric acid is used for acid hydrolysis, which can be easily removed from the medium after the incubation period. Excessive concentrations of strong acids lead to the reduction in monomeric sugars and inhibition of fermentation by producing toxic compounds (Agustini et al. 2019). Therefore, diluted sulfuric acid (0.5M) was used in this study. The duration of the hydrolysis process is also an important factor. Penetrating cells for a long time may reduce the bioethanol yield. (Harun et al. 2009). Acid hydrolysis is effective at the temperature range between 100- 200 °C. Hence, 60ml/100mL of sulfuric acid was chosen for further studies.

3.4 Effect of nitrogen source

When different nitrogen sources such as soybean flour, corn-flour, peptone, and mung bean flour were used in the fermentation media, significantly higher ethanol production (1.53%) was witnessed in the medium containing soybean (Figure 03) than the other nitrogen sources. Soybean is rich in nitrogen content. Organic & inorganic nitrogen sources in the fermentation medium increase the growth of *S. cerevisiae* (Marti and Olmo, 2008).

3.5 Effect of the concentration of the nitrogen source

When the amount of soybean powder was used as 1.0g/100ml, the ethanol yield was significantly increased by 1.54 times (from 1.50% to 2.30%, Figure 04) than the non-optimized amount of soybean powder (1.5g/100ml). Fermentation medium containing 1.0g of soybean powder yielded significantly higher ethanol production than other concentrations. A higher concentration of nitrogen may inhibit the growth of yeast in the fermentation medium & this will lead to a decrease in ethanol production. Hence 1.0g/100ml of nitrogen source (soybean flour) in the fermentation media was chosen for further studies.

Nitrogen supplements are mandatory for the enhancement of growth of yeast cells (Marti and Olmo, 2008). Adequate amounts of nitrogen should be used to supplement the medium. Suitable nitrogen sources can reduce the formation of inhibitory by-products and this increases the bioethanol yield (Adnan, et al., 2014). Lack of nitrogen supplements leads to less yeast biomass production which can result in sluggish fermentation. This reduces the bioethanol yield (Henschke and Jiranek. 1993; Alexendra and Charpentier. 1998).

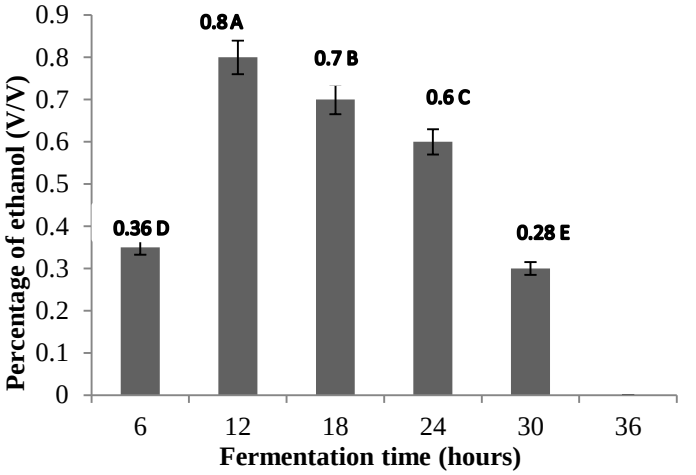

Figure 02: Effect of different incubation periods on bioethanol production from papaw fruit peel mixture using *Saccharomyces cerevisiae*.
(Different alphabetical letters show significant differences between the mean values)

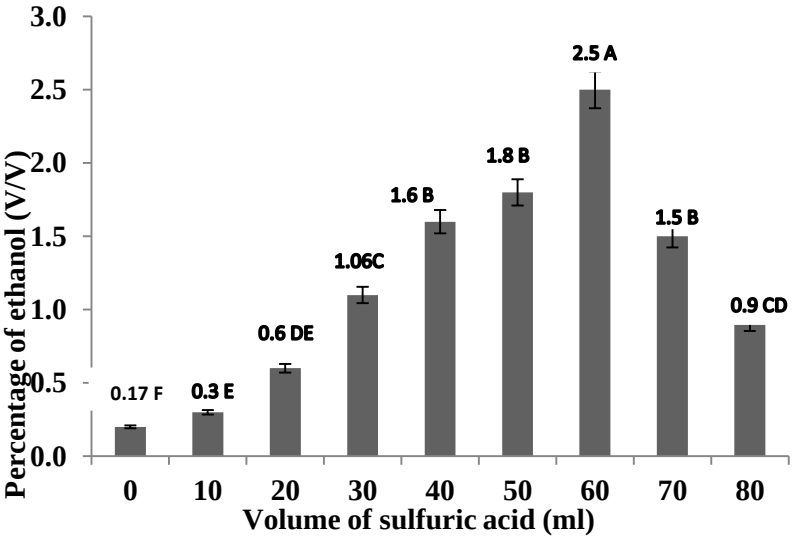

Figure 03: Effect of volume of sulfuric acid on bioethanol production from papaw peel mixture using *Saccharomyces cerevisiae*.
(Different alphabetical letters show significant differences between the mean values)

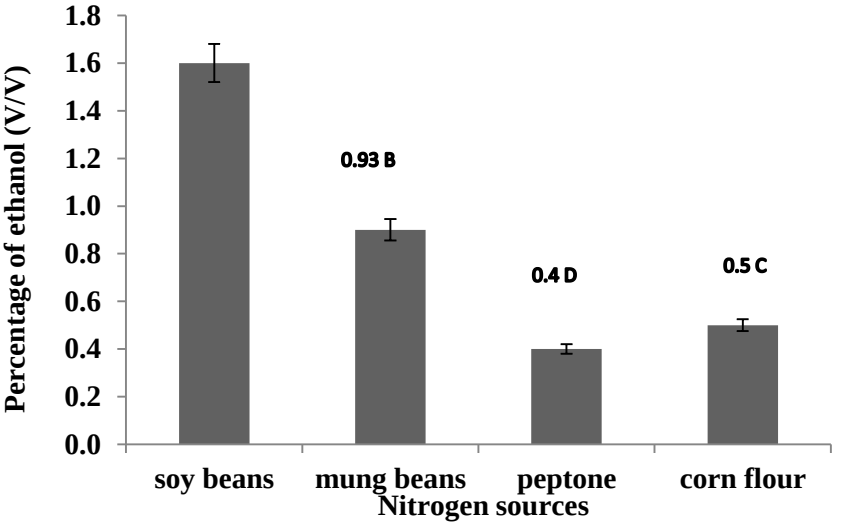

Figure 04: Effect of nitrogen sources on bioethanol production from papaw fruit peel using *Saccharomyces cerevisiae*.
(Different alphabetical letters show significant differences between the mean values)

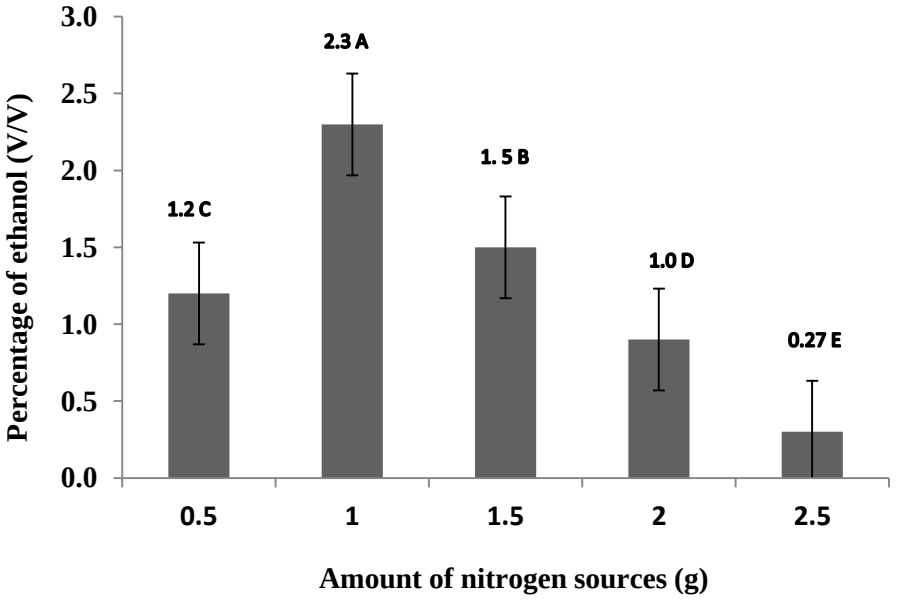

Figure 05: Effect of different amount of nitrogen sources on bioethanol production from papaw fruit peel using *Saccharomyces cerevisiae.*
(Different alphabetical letters show significant differences between the mean values.)

3.6 Effect of ratio of the fermenting solution: air space

When different ratio of volumes of fermentation solutions (50ml, 100ml, 150ml, 200ml, and 250ml) were chosen, significantly higher ethanol production was obtained with 100ml of fermentation solution (1.25 times, from 0.80% to 1.00%) than the non-optimized fermentation solution of 200ml (Figure 05). Volume of fermentation solution has a direct effect on fermentation rate and microbial cells as this determines the ratio of air space in the fermenting solution. Generally, this ratio expresses the amount of oxygen present in the media.

Fermentation of simple sugars into ethanol by yeast cells is an anaerobic process. But yeast cells have a potential to take up the oxygen. Presence of larger amount of oxygen (if the ratio is high) in the medium can lead the yeast cells engaging in aerobic respiration process. This leads to complete fermentation of sugars with decreased amount of ethanol yield and the release of carbon dioxide. Also, if the oxygen content is too low (if the ratio is low) this can reduce the viability of the yeast cells (Rosenfeld et al. 2003).

3.7 Effect of inoculum size

When different amounts of yeast inoculum (0.5g, 1.0g, 1.5g, 2.0g, 2.5g) were chosen, significantly higher ethanol production was obtained with 1.5g of the amount of inoculum (1.2 times, from 1.00% to 1.20%) than the non-optimized amount of substrate of 1.0g (Figure 07). The concentration of added inoculum in the fermentation media does not have a significant influence on final ethanol production, but it affects the sugar consumption rate by yeast cells (Laopaiboon et al. 2007). Hence 1.5 g/100mL of yeast inoculum was chosen for further studies. When yeast inoculum is increased cells grow rapidly with increase in fermentation time. This results in the higher ethanol production. When the inoculum size reaches a certain range of ethanol production - its maximum level - ethanol production can be decreased. This is due to the depletion of nutrients and immediate consumption of sugars by yeast cells (Hosny, et al. 2016).

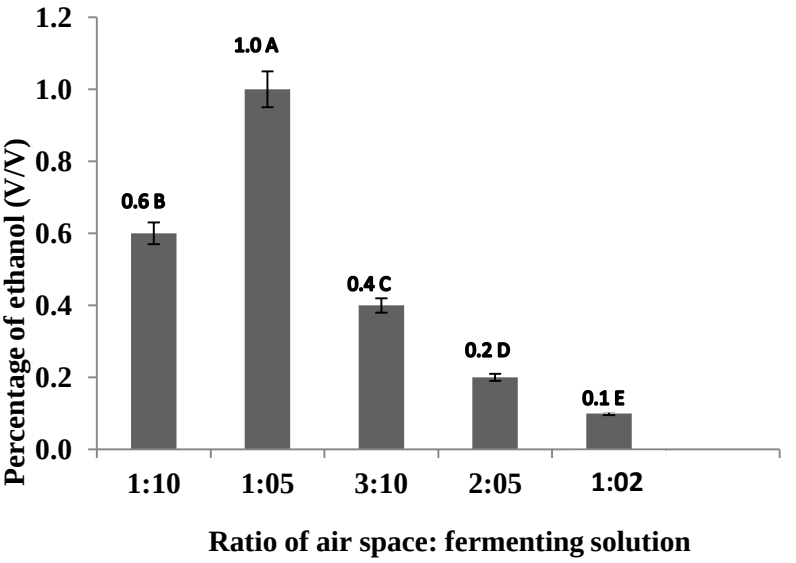

Figure 06: Effect of ratio of air space: solution on bioethanol production from papaw fruit peel mixture using *Saccharomyces cerevisiae.*
(Different alphabet letters show significant differences between the mean values.)

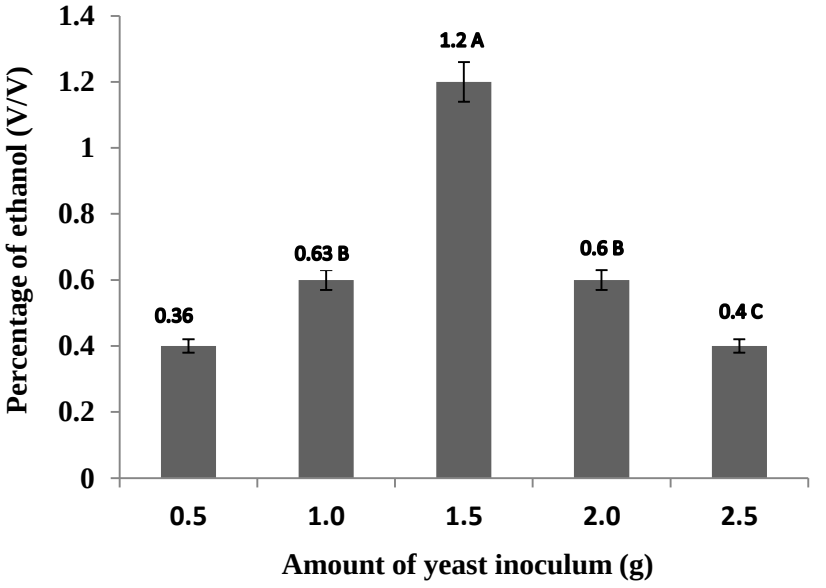

Figure 07: Effect of different size of inoculum on bioethanol production from Papaw fruit peel juice using *Saccharomyces cerevisiae.*
(Different alphabet letters show significant differences between the mean values.)

3.8 Effect of amount of substrate (raw fruit peel mixture)

When different amounts of raw fruit peel mixture (5g, 10g, 15g, 20g and 25g) were chosen, significantly higher ethanol production was obtained with 15.0g of the amount of substrate (1.25 times, from 1.20% to 1.50%) than the non-optimized amount of substrate of 10g (Figure 07). The amount of substrate has a direct effect on fermentation rate and microbial cells. Generally, the fermentation rate is going to be enlarged with the rise in substrate concentration up to a definite level. However, the surplus sugar concentration can exceed the uptake capability of the cells of microorganisms resulting in a gradual rate of fermentation. Ethanol production can reach a higher level according to the initial sugar concentration used (Laopaiboon et al, 2007). Hence 15.0g substrate in the fermentation media was chosen for further studies.

3.9 Effect of pH of the medium

When the pH of the media was kept at 5.0, the ethanol yield was significantly increased by 1.48 times (from 2.50% to 3.70%) than the non-optimized control pH 6.0 (Figure 08). The management of pH have a direct influence on the growth of microorganisms used for the fermentation process and conjointly on their cellular processes (Kasemets et al. 2007; Pirselove et al. 1993). The H+ concentrations in the fermentation broth will be ready to amend the entire charge of the plasma membrane as to moving the porosity of some essential nutrients into the cells. Once the fermentation medium becomes more acidic, the fermentation rate conjointly will increase. Enzymes made by yeast to ferment aldohexose may need customary conditions made for acidic conditions. Yeast cells are more tolerant to acidic conditions than basic conditions. The organic and inorganic chemicals employed in the media may be responsible for the change in the pH of the media due to the different ions released. Hence, pH of the fermentation media was chosen as 5.0 for further studies.

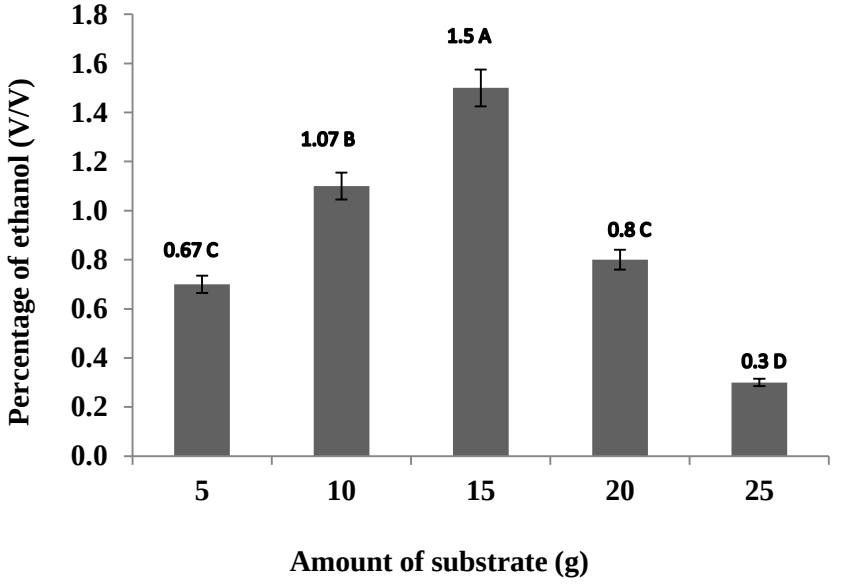

Figure 08: Effect of different amounts of fruit peel mixture on bioethanol production from papaw fruit peel juice using *Saccharomyces cerevisiae.*
(Different alphabetical letters show significant differences between the mean values.)

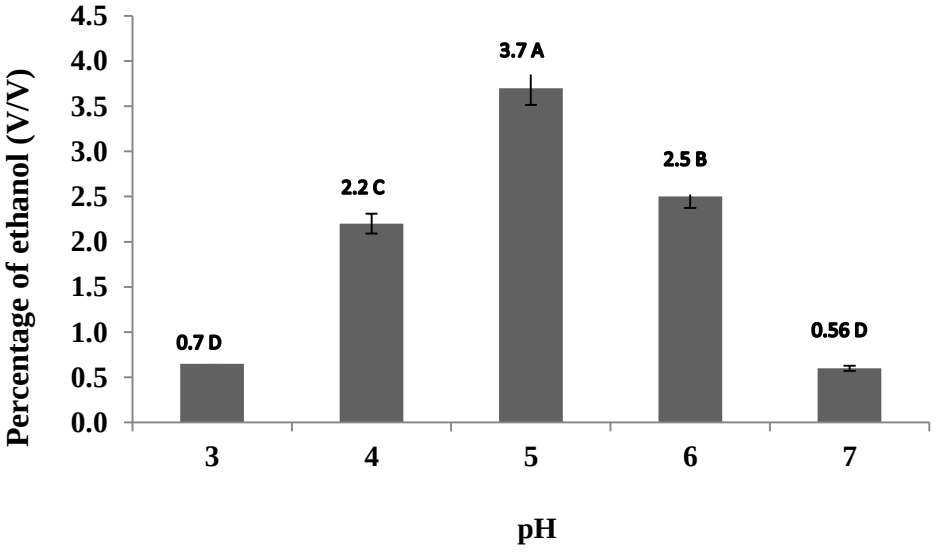

Figure 09: Effect of different pH on bioethanol production from papaw fruit peel mixture using *Saccharomyces cerevisiae.*
(Different alphabet letters show significant differences between the mean values.)

3.10 Anti -bacterial activity

The results revealed that the ethanol that is produced using the fermentation of papaw peel can efficiently suppress the growth of selected bacterial strains which are considered as human pathogenic microorganisms. A maximum zone of inhibition was observed within the Escherichia coli (15.4mm) (Figure 09). The different hypothesis suggests different mechanisms of anti-bacterial action. Organic compounds are bounded (by hydrogen bonding and hydrophobic bonding) to the protein molecules that are found in the biological membranes. This bonding is followed by the partition in the lipid bilayer. Perturbation of membrane permeability is consequent to its expansion and elevated fluidity by causing the inhibition of enzyme in the embedded membrane. Furthermore, this causes disruption of biological membranes and destruction of electron transportation and perturbation of the cell wall. Ethanol has the capacity of killing bacterial strains by the process of denaturation (Kapilan & Anpalagan, 2015). Alcohol molecules bind with the fat membrane of the bacterial cell and the cell are consequently vulnerable. This leads to the leakages of bacterial cells that finally results in their death (Ingram. 1990, Kapilan & Thavaranjit. 2009).

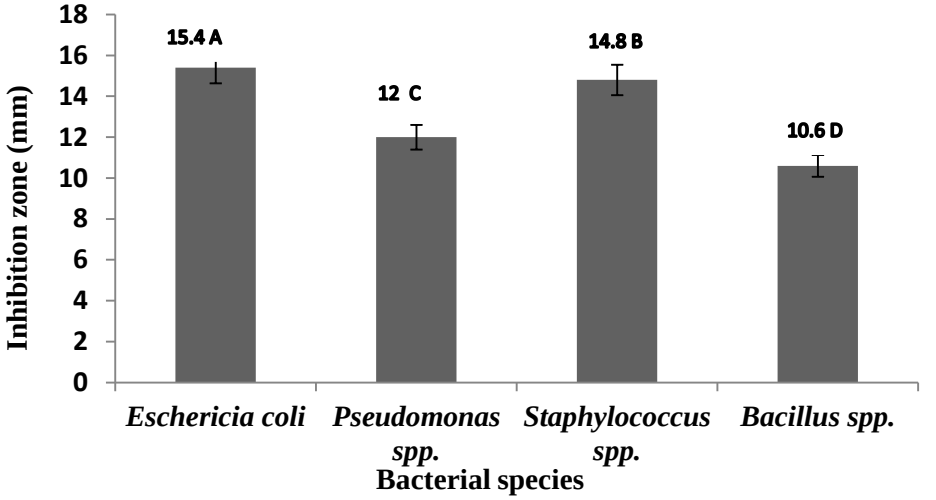

Figure 10: Antibacterial activity of ethanol against selected human pathogens. (Different alphabetical letters show significant differences between the mean values.)

4 CONCLUSIONS

The *Carica papaya* (papaw) peel waste is an effective substrate for bioethanol production using baker's yeast. After optimization of nitrogen sources, culture conditions, and media composition, the bioethanol yield was significantly increased (6.2 times, from 0.60% to 3.70%) than the non-optimized conditions. All the bacterial strains that were used for this study *(Escherichia coli, Staphylococcus aureus, Pseudomonas spp., and Bacillus spp.)* showed inhibition

zones thus, they were sensitive for the bioethanol extract. Fungal and viral responses of the bioethanol extract require to be studied to determine whether this bioethanol extract could be used as a potential hand sanitizer.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to National Research Council of Sri Lanka, for the financial support.

REFERENCES

Adnan, NAA, Suhaimi, SN, Abd-Aziz, S, Hassan, MA & Phang, LY 2014, 'Optimization of bioethanol production from glycerol by *Escherichia coli* SS1', *Renewable Energy,* Vol. 66, pp. 625-633. Available from: https://doi.org/10.1016/j.renene.2013.12.032 [June 2014]

Alexandre, H & Charpentier, C 1998, 'Biochemical aspects of stuck and sluggish fermentation in grape must', *J. Ind. Microbiol. Biotechnol*. Vol. 20, pp. 20–27. https://doi.org/10.1038/sj.jim.2900442

Agustini, NWS, Hidhayati, N & Wibisono, SA 2019, 'Effect of hydrolysis time and acid concentration on bioethanol production of microalga *Scenedesmus sp.*', *Earth and environmental Science,* Available from: https://iopscience.iop.org/article/10.1088/1755-1315/308/1/012029

Aron, NSM, Khoo, KS, Chew KW, Show PL, Chen, WH & Nguyen THP 2020, 'Sustainability of the four generations of biofuels - A review', *International journal of energy research,* vol.44, no. 12, pp. 9266-9282. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/er.5557 [15 June 2020].

Asmamaw, T & Fassil, AC, 2014, 'Trends in Bioethanol Production by *Saccharomyces cerevisiae:* Substrate, Inhibitor Reduction, Growth Variables, Co-culture, and Immobilization', *International Scholarly Research Notices,* Available from: https://doi.org/10.1155/2014/532852

Azhar, SHM, Abdulla, R, Jambo, SA, Marbawi, H, Gansau, JA, Faik, AAM & Rodrigues, KF 2017, 'Yeasts in sustainable bioethanol production: A review', *Biochemistry and Biophysics Reports*, vol. 6, pp. 52- 61. Available from: https://doi.org/10.1016/j.bbrep.2017.03.003 [06 March 2017]

Berardi, A, Perinelli, DR, Merchant, HA, Bisharat, L, Basheti, IA, Bonacucina, G, Cespi, M & Palmieri, GF 2020, 'Hand

sanitisers amid CoViD-19: A critical review of alcohol-based products on the market and formulation approaches to respond to increasing demand', *Elsevier Public Health Emergeny Collection*, Available from: https://pubmed.ncbi.nlm.nih.gov/32461194/ [16 May 2020].

Bušić, A, Marđetko, N, Kundas, S, Morzak, G, Belskaya, H, Šantek, MI Komes, D, Novak, S & Šantek, B 2018, 'Bioethanol Production from Renewable Raw Materials and Its Separation and Purification', *Food technology & biotechnology,* vol. 56, no. 3, pp. 289–311. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6233010/

Chitranshi, R & Kapoor, R 2021, 'Utilization of over-ripened fruit (waste fruit) for the ecofriendly production of ethanol', *Vegetos*, vol. 4, pp. 270- 276. Available from: https://link.springer.com/article/10.1007/s42535-020-00185-8 [05 Feruary 2021]

Goh, CS & Lee, KT 2010, 'Palm-based biofuel refinery (PBR) to substitute petroleum refinery: An energy and emergy assessment', *Renewable and Sustainable Energy Reviews,* vol. 14, no. 9, pp. 2986-299. Available from: https://doi.org/10.1016/j.rser.2010.07.048

Gold, NA, Mirza, TM, & Avva, U 2021, 'Alcohol Sanitizer', *Star Pearl publishing, Treasure Island (FL),* Jan 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513254/ [26th June 2021]

Golin, AP, Choi, D & Ghahary, A 2020, 'Hand sanitizers: A review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses', *American Journal of Infection Control,* vol. 48, no. 9, pp. 1062-1067. Available from: https://doi.org/10.1016/j.ajic.2020.06.182 [18th June 2020].

Hakimzadeh, V, Razavi, SMA, Piroozifard, MK, and Shahidi, M 2006, 'The potential of microfiltration and ultrafiltration process in purification of raw sugar beet juice', *Desalination,* vol. 200, no.1, pp. 520–522. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0011916406007648 [20 November 2006].

Hans, M., Lugani, Y & Chandel, AK 2021, 'Production of first- and second-generation ethanol for use in alcohol-based

hand sanitizers and disinfectants in India', *Biomass Conversion and Bio refinery*. Available from: https://doi.org/10.1007/s13399-021-01553-3 [27 May 2021].

Harun, R, Danquah, MK, & Forde, GM 2009, 'Microalgal biomass as a fermentation feedstock for ethanol production', *Journal of chemical technology and biotechnology,* vol. 85, no.2, pp. 199-203. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/jctb.2287 [04 November 2009]

Henschke, P & Jiranek, V 1993, 'Yeasts-metabolism of nitrogen compounds in wine microbiology and biotechnology', *Aust. Wine Res. Inst*, pp. 77–164.

Hosny, M, Abo-State, MA, El-Temtamy, SA & El- Sheikh, HH 2016, 'Factors Affecting Bioethanol Production from Hydrolyzed Bagasse', *International Journal of Advanced Research in Biological Sciences,* vol. 3, no. 9, pp. 130-138. Available from: http://dx.doi.org/10.22192/ijarbs.2016.03.09.019 [09th March 2016]

Hossain, ABMS, Ahmed, SA, Ahmed, M, AlshammariFaris, M, Adnan, A, Annuar, MSM, Mustafa, H & Norah, H 2011, 'Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy', *African Journal of Microbiology Research*, vol. 5, no.6, pp. 586-598. Available from: http://www.academicjournals.org/ajmr [18 March 2011].

Ingram, LO 1990, 'Ethanol tolerance in bacteria', *Critical Reviews in Biotechnology,* vol. 9, no. 4, pp. 305-319. Available from: https://pubmed.ncbi.nlm.nih.gov/2178781/

'Impact of COVID-19 on Hand Sanitizer Market Size, Share, Industry Analysis and Regional Forecast, 2019-2026' 2021, *Fortune Business Insights*, 08 April . Available from: https://www.globenewswire.com/news-release/2021/04/08/2206514 [08th April 2021]

Iranmahboob, J, Nadim, F, Monemi, S 2002, 'Optimizing acid-hydrolysis: a critical step for production of ethanol from mixed wood chips', *Biomass Bioenergy,*vol. 22, no. 5, pp. 401-404. Available from:

https://doi.org/10.1016/S0961-9534(02)00016-8

Jahid, M., Gupta, A and Sharma, DK 2018, 'Production of Bioethanol from Fruit Wastes (Banana, Papaya, Pineapple and Mango Peels) Under Milder Conditions', *Journal of Bioprocessing & Biotechniques,* vol. 8, no. 3. Available from: https://www.hilarispublisher.com/open-access/production-of-bioethanol-from-fruit-wastes-banana-papaya-pineapple-and-mango-peels-under-milder-conditions-2155-9821-1000327.pdf [14 May 2018].

Jing, JLJ, Yi, TP, Bose, RJC, McCarthy, JR, Tharmalingam, N & Madheswaran, T 2020, 'Hand Sanitizers: A Review on Formulation Aspects, Adverse Effects, and Regulations', *International Journal of Environmental Research and Public Health,* Vol.17, no. 9, pp. 3326. Available from: https://dx.doi.org/10.3390%2Fijerph17093326 [11th May 2020]

Kaewkrajay, C, Dethoup, T & Limtong, S 2014, 'Ethanol production from cassava using a newly isolated thermo tolerant yeast strain', *ScienceAsia,* vol. 40,pp. 268–27. Available from: https://doi.org/10.2306/scienceasia1513-1874.2014.40.268 [12th May 2014]

Kapilan, R & Anpalagan, VC 2015, 'Antimicrobial activity and amylase production by *Rhizophus microsporus* strain isolated from germinating maize seed', *Asian Journal of Plant Science and Research,* vol. 5, no. 10, pp. 01-08. Availale from: https://www.imedpub.com/abstract/

Kapilan, R & Thavaranjit, AC 2009, 'Antimicrobioal activity of *Trichoderma polysporum', Journal of science of Eastern University of Sri Lanka,* vol. 6, no.1, pp. 78-87. Available from: https://www.fsc.esn.ac.lk/jsc/archive/6.9.pdf

Kasemets, K, Nisamedtinov, I, Laht, TM, Abner, K & Paalme, T 2007, 'Growth characteristics of *Saccharomyces cerevisiae* S288C in changing environmental conditions: auxo-accelerostat study', *Antonie van Leeuwenhoek,* vol. 92, no. 1, pp. 109–128. Available from: https://link.springer.com/article/10.1007/s10482-007-9141-y [01 February 2007].

Kawa-Rygielska, J, Pietrzak, W, Regiec, P & Stencel, P 2013, 'Utilization of

concentrate after membrane filtration of sugar beet thin juice for ethanol production', *Bioresource Technology*, vol. 133, pp. 134–141. Available from: https://doi.org/10.1016/j.biortech.2013.01.070 [April 2013].

Khoja, AH, Ali, E, Zafar, K, Ansari, AA, Nawar, A & Qayyum, M 2015, "Comparative study of bioethanol production from sugarcane molasses by using *Zymomonas mobilis* and *Saccharomyces cerevisiae', African Journal of Biotechnology*, vol. 14, no. 31, pp. 2455-2462. Available from: http://www.academicjournals.org/AJB [5 August 2015]

Kosaric, N & Velikonja, J 1995, 'Liquid and gaseous fuels from biotechnology: challenge and opportunities', *FEMS Microbiology Reviews*, vol. 16, no, 2-3, pp. 111–142, Available from: https://doi.org/10.1111/j.1574-6976.1995.tb00161.x [01 February 1995]

Laopaiboon, L, Thanonkeo, P, Jaisil, P & Laopaiboon, P 2007, 'Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae', World Journal of Microbiology and Biotechnology*, vol. 23, no. 10, pp. 1497–1501. Available from: https://link.springer.com/article/10.1007%2Fs11274-007-9383-x [06 April 2007].

Marti, EJ &Olmo, MD 2008, 'Addition of ammonia or amino acids to a nitrogen-depleted medium affects gene expression patterns in yeast cells during alcoholic fermentation ',*FEMS Yeast Research*, vol. 8, no. 2, pp. 245–256: Available from: https://doi.org/10.1111/j.1567-1364.2007.00325.x [01 March 2008]

Miller, GL 1959, 'Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar', *Analytical chemistry*, vol. 31, no. 3, pp. 426–428. Available from: https://doi.org/10.1021/ac60147a030

Nadir, N, Mel, M, Karim, MIA & Yunus, RM 2009, 'Comparison of sweet sorghum and cassava for ethanol production by using *Saccharomyces cerevisiae', Journal of Applied Sciences*, vol. 9, no. 17, pp. 3068–3073. Available from: https://dx.doi.org/10.3923/jas.2009.3068.3073

Olsson, L & Hahn-Hägerdal, B 1993, 'Fermentative performance of bacteria and yeasts in lignocellulose hydrolysates', *Process Biochemistry*, vol. 28, no. 4, pp. 249–257. Available from:

https://doi.org/10.1016/0032-9592(93)80041-E

Omar, G, Abdallah, L, Ismail, S & Almasri, M 2013, 'Screening of Selected Medicinal Wild Plant Extracts Antibacterial Effect as Natural Alternatives', *International Journal of Indigenous Medicinal Plants*, vol. 46, no. 2, pp. 1299-1304. Available from: https://www.researchgate.net/publication/257937527_Screening_of_Selected_Medicinal_Wild_Plant_Extracts_Antibacterial_Effect_as_Natural_Alternatives [August 2013].

Piršelová, K, Šmogrovičová, D & Baláž, S 1993, 'Fermentation of starch to ethanol by a co-culture of Saccharomycopsis fibuligera and *Saccharomyces cerevisiae'*, *World Journal of Microbiology & Biotechnology*, vol. 9, no. 3, pp. 338–341. Available from: https://link.springer.com/article/10.1007/BF00383075 [May 1993].

Regiec, P 2004, 'Purification of diffusion juice with ultrafiltration ceramic membrane', *ActaAgrophysica*, vol. 4, no. 2, pp. 491–500. Available from: http://www.acta-agrophysica.org/Purification-of-diffusion-juice-with-ultrafiltration-ceramic-membrane,108144,0,2.html

Romella, FD, Rani, A & Manohar RS 2016, 'Chemical composition of Some selecte fruit peels', *European Journal of Food Science and Technology* , vol.4, no. 4, pp. 12-21. Available from: https://www.researchgate.net/publication/326579276_Chemical_composition_of_some_selected_fruit_peels

Rosenfeld, E, Beauvoit, B, Blondin, B & Salmon, JM 2003, 'Oxygen consumption by anaerobic Saccharomyces cerevisiae under enological conditions: effect on fermentation kinetics', *Applied Environmental Microbiology*, vol. 69, no. 1, pp. 113-21. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC152411/

Shahidi, M & Razavi, SMA 2006, 'Improving thin sugar beet juice quality through ultrafiltration', *Desalination*, vol. 200, no. 1, pp. 518–519. Available from: https://profdoc.um.ac.ir/paper-abstract-202459.html

Sikarwar, V, Zhao, M, Fennell, PS & Shah, N 2017, 'Progress in biofuel production from gasification', *Progress in Energy and Combustion Science*, vol. 61, pp. 189- 248.

Available from: https://doi.org/10.1016/j.pecs.2017.04.001

Singh, D, Joshi, K, Samuel, A, Patra, J & Mahindroo, N 2020, 'Alcohol-based hand sanitisers as first line of defence against SARS-CoV-2: a review of biology, chemistry and formulations', *Epidemiology & Infection* , Vol. 148 , e229. Available from: DOI: https://doi.org/10.1017/S0950268820002319

Singh, R, Prakash, A, Balagurumurthy, B, & Bashkar, T 2015, 'Hydrothermal Liquefaction of Biomass', *Recent Advances in Thermo-Chemical Conversion of Biomass,* pp. 269-291. Available from: https://doi.org/10.1016/B978-0-444-63289-0.00010-7

Tamers, M 2006, 'Distinguishing between bio-ethanol and petroleum-ethanol', *Ethanol producer magazine.* Available from: http://ethanolproducer.com/articles/2077/distinguishing-between-%EF%BF%BDbio-ethanol'-and-petroleum-ethanol [01 June 2006].

The state of food security and Nutrition in the World 2021, *Food and Agriculture Organization of United Nations,* 2020. Available from: http://www.fao.org/state-of-food-security-nutrition

United States Food & Drug Administration, 2020, 'Q&A for Consumers | Hand Sanitizers and COVID-19' Available from: https://www.fda.gov/drugs/information-drug-class/qa-consumers-hand-sanitizers-and-covid-19 [15th December 2020]

Vikramaditya, G, Yadav, G V, Yadav, GD & Patankar, SC 2020, 'The production of fuels and chemicals in the new world: critical analysis of the choice between crude oil and biomass vis-à-vis sustainability and the environment', *Clean Technologies and Environmental Policy*, vol. 22, pp. 1757–1774. Available from: https://link.springer.com/article/10.1007%2Fs10098-020-01945-5 [21 September 2020].

Vohra, M, Manwar, J, Manmode, R, Padgilwar, S & Patil, S 2013, 'Bioethanol production: Feedstock and current technologies', *Journal of environmental chemical engineering,* vol. 2, no. 1, pp. 573- 584. Available from: http://dx.doi.org/10.1016/j.jece.2013.10.013

Youcai, Z., & Tao, Z. (2021). Chapter 1 - Anaerobic fermentation process for biohydrogen production from food waste. *Biohydrogen Production and Hybrid Process Development:* pp. 1-24. Available from: https://doi.org/10.1016/B978-0-12-821728-3.00001-3

Zabed, H, Faruq, G, Sahu, JN & Azirun, MS 2014, 'Bioethanol Production from Fermentable Sugar Juice', *The Scientific World Journal.* Availale from: http://dx.doi.org/10.1155/2014/957102 [12th March 2014]

Zoumpourlis, V, Goulielmaki, M, Rizos, E, Baliou, S & Spandidos, DA 2020, 'The COVID-19 pandemic as a scientific and social challenge in the 21st century', *Molecular Medicine Reports,* vol. 22, no. 4, pp. 3035–3048. Available from: https://doi.org/10.3892/mmr.2020.11393 [30 July 2020].