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Fermentation of Waste Fruits for Bioethanol Production

[1]Shubhra Tiwari, [1]S.K. Jadhav, [1]Mayuri Sharma and [2]K.L. Tiwari
[1]School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India
[2]Department of Biotechnology, Gurughasi Das University, Bilaspur, Chhattisgarh, India

Corresponding Author: Shubhra Tiwari, School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India Tel: +919165716883 Fax: 07712262583

ABSTRACT

Dwindling supplies of petroleum and growing environmental concerns over its use has led to increasing interest in developing biomass as a feedstock for liquid fuels. In particular, bioethanol produced from biomass represents a promising alternative fuel or gasoline extender. Bioethanol is used in vehicles either as a sole fuel or blended with gasoline. As an oxygenated compound, ethanol provides additional oxygen in combustion and hence obtains better combustion efficiency. The main environmental advantages of fuel ethanol are its sustainability in using a renewable resource as a feedstock, thus promoting independence of fossil fuel and maintaining the level of greenhouse gas. Bioethanol can be produced from carbohydrate containing substrates by the process of fermentation. Many microorganisms like bacteria, fungi and yeast involved in bioethanol production. Present study deals with bioethanol production from rotten fruits with inoculation of bacteria isolated from respective fruits. Rotten fruits serve as potential feedstock for bioethanol production due to high sugar content and cost effective substrate. Results indicate that among 5 fruits rotten sapota (*Manilkara zapota*) produced highest amount of bioethanol 9.40% on 5th day of incubation. Bioethanol can solve the problem of pollution and considered as fuel for future.

Key words: Fermentation, fungi, bacteria, bioethanol, biofuel

INTRODUCTION

The world's reliance on fossil fuels for transport is unsustainable. In addition, fossil fuels are the main reason for global warming, a process that practically all climate scientists say we have to deal with not soon, not tomorrow, but now. One of the most promising alternate source of energy is bioethanol. Bioenergy represents the utilization of biomass as starting material for the production of sustainable fuels and chemicals (Fukuda *et al.*, 2009). Ethanol has long been considered as a suitable alternative to fossil fuels either as a sole fuel in cars with dedicated engines or as an additive in fuel blends with no engine modification requirement when mixed upto 30%. Today, bioethanol is the most dominant biofuel and its global production showed an upward trend over the last 25 years with a sharp increase from 2000 (Talebnia *et al.*, 2010). Sugar and starch based materials such as sugarcane and grains are 2 groups of raw materials currently used as the main resources for ethanol production. The 3rd group is lignocellulosic materials representing the most viable option for production of ethanol. Growing demand for human food, as it is for energy and considering the priority for starving human society could make the first 2 groups of raw materials potentially less competitive and perhaps expensive feed stocks in the near future compared to lignocellulosic materials (Taherzadeh and Karimi, 2007). The bio-fuels to be considered as relevant technologies by both developing and industrialized countries are due to a number of factors, including energy security reasons, environmental concerns, foreign exchange savings and socioeconomic issues related to the rural sector. Increasing use of biofuels for energy generation purposes is of particular interest nowadays because they allow mitigation of greenhouse gases, provide means of energy independence and may even offer new employment possibilities (Wierzbicka *et al.*, 2005). Biofuels are non-toxic, biodegradable and free of sulphur and carcinogenic compounds like benzene (Sastry *et al.*, 2006). Biofuels are being investigated as potential substitutes for current high pollutant fuels obtained from conventional sources (Nwafor, 2004). Biofuels are liquid or gaseous fuels made from plant matter and residues, such as agricultural crops, municipal wastes and agricultural and forestry by-products.

MATERIAL AND METHODS

Collection of substrate: Different rotten fruits (Sapota, papaya, apple, banana and grapes) were collected from local market.

Isolation of bacteria from different sources: All the rotten fruits were dipped in distilled water separately (50 g in 500 mL). Their sap was poured in nutrient agar medium. Bacteria obtained from rotten apple, berry, grapes, papaya and sapota was named as bacteria A, B, G, P and S, respectively and their microscopic characters were studied.

Fermentation test: Fermentation test was performed to check whether the obtained bacteria were fermentative or not. It was carried out in a tube which contains a durham tube placed in inverted position. The composition of fermentation broth was peptone 10 g, sodium chloride 15 g, carbohydrate 5 g, phenol red 0.018 g and 1000 mL of distilled water (Taras and Muzio, 2002).

Inoculation of fermentative bacteria into substrate: All 5 rotten fruits were dipped into distilled water (50 g in 500 mL) and autoclaved. All respective fermentative bacteria were inoculated into rotten fruit substrate and kept for incubation for 48 h at 37°C in incubator.

Qualitative estimation of bioethanol: Bioethanol production was examined by Jones reagent [$K_2C_{r2}O_7$+H_2SO_4] 1 mL of $K_2C_{r2}O_7$ (2%), 5 mL H_2SO_4 and 3 mL of sample was added after incubation. It was reported that ethanol oxidized to acetic acid with an excess of potassium dichromate in the presence of sulfuric acid, giving off a blue-green colour (Brooks, 2008). The presence of a green colour indicates that the used carbon source was able to produce bioethanol after confirmation.

Quantitative estimation of bioethanol: Quantitative estimation of bioethanol was done by specific gravity method. Specific gravity refers to the density of any liquid (Government of India, 1985).

The fermented sample 25 mL was taken and 150 mL of distilled water was added this mixture was distilled on distillation unit.

After distillation of sample, percentage of bioethanol was calculated by specific gravity method (Yadav, 2003).

RESULTS AND DISCUSSION

Present study deals with bioethanol production from different rotten fruits which are wasted in market and godowns. Five rotten fruits such as sapota, papaya, apple, banana and grapes were selected for bioethanol production as they are good source of carbohydrate naturally. Qualitative estimation of bioethanol was done by method of Jones reagent test and estimation of bioethanol production done by specific gravity method. Bacteria were isolated from respective 5 rotten fruits named as A, B, G, P, S and inoculated in fruit samples to study the bioethanol production. Microscopic observation of bacteria shows that bacteria A was coccus, bacteria B was coccus, bacteria G was bacillus, bacteria P was coccus and bacteria S was bacillus. All 5 bacteria were motile, bearing endospore, acid fast negative and bacteria A was gram negative and other bacteria B, G, P and S were gram positive.

In rotten fruit apple produced 6.15% of bioethanol on 3rd day, in 4th day production of bioethanol was 7.22, 5.88 and 4.81%, respectively for 5th and 6th day. Maximum amount of bioethanol was produced on 4th day (Table 1). Vendruscolo *et al*. (2008) used apple pomace as versetile substrate for bioethanol production. Neelakandan and Usharani (2009) also study bioethanol production from cashew apple juice using *Saccharomyces cerevisiae*. Rotten berry produced 4.81% of bioethanol on 3rd day, 5.08% on 4th day, 7.00% on 5th and 3.18% on 6th day of incubation (Table 2). After the fermentation of rotten grapes sample percentage of bioethanol production was 5.36, 8.04, 7.76 and 7.49% on 3rd-6th day of incubation, respectively. The maximum production of bioethanol was obtained on 4th day of incuabation (Table 3). Korkie *et al*. (2002) worked on grape pomace for ethanol production. Rotten papaya obtained the 2.64% of bioethanol on 3rd day, 3.18% on 4th day, 6.41% on 5th day and 2.37% on 6th day. Production of

Table 1: Amount of bioethanol produced by apple

Incubation period (day)	Amount of bioethanol (%)
3rd	6.15
4th	7.22
5th	5.88
6th	4.81

Table 2: Amount of bioethanol produced by berry

Incubation period (day)	Amount of bioethanol (%)
3rd	4.81
4th	5.08
5th	7.00
6th	3.18

Table 3: Amount of bioethanol produced by grapes

Incubation period (day)	Amount of bioethanol (%)
3rd	5.36
4th	8.04
5th	7.76
6th	7.49

Table 4: Amount of bioethanol produced by papaya

Incubation period (day)	Amount of bioethanol (%)
3rd	2.64
4th	3.18
5th	6.41
6th	2.37

Table 5: Amount of bioethanol produced by sapota

Incubation period (day)	Amount of bioethanol (%)
3rd	6.68
4th	7.49
5th	9.40
6th	8.04

bioethanol was maximum on day of 5 (Table 4). Akin-Osanaiye *et al.* (2008) produced bioethanol form *Carica papaya* waste. Sharma *et al.* (2007) explained optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels. Rotten sapota produced 6.68% of bioethanol on 3rd day, 7.49% of bioethanol on 4th day, 9.40% of bioethanol on 5th day and 8.04% of bioethanol on 6th day (Table 5). Result shows that highest production of bioethanol was obtained on 5th day of incubation. Tiwari *et al.* (2010) studied effect of temperature variations in bioethanol production production process. Tiwari *et al.* (2011) also explained bioethanol production from some carbohydrate sources by gram positive bactetia. Tiwari *et al.* (2012) worked on Jatropha oil cake for bioethanol production. Pandey *et al.* (2013) used Azolla a lignocellulosic waste for bioethanol production.

CONCLUSION

In current time the importance of alternative energy source has become even more necessary not only due to the continuous deletion of limited fossil fuel stock but also for the safe and better environment. Rotten fruits may serve as a good substrate as they contains sufficient amount of carbohydrates naturally which can be used for production of bioethanol. Among all used fruits sapota gave maximum production of bioethanol. Bioethanol consider as a fuel of the future and can solve the problem of pollution and energy crisis.

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