Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Elixir Appl. Botany 48 (2012) 9612-9615

ISSN: 2229-712X

A new technology for extraction of Palm sap from Palmyrah tree for Bioethanol production

Sivalingam Arunavathi[1], Peramathi Palanivelu[2] and Kasinadar Veluraja[1,*]

[1]Department of Physics, Manonmaniam Sundaranar University, Tirunelveli – 627 012, TamilNadu, India.

[2]Department of Molecular Microbiology, School of Biotechnology, Madurai Kamaraj University, Madurai, TamilNadu, India.

ARTICLE INFO

Article history:

Received: 2 April 2012;
Received in revised form: 28 June 2012;
Accepted: 25 July 2012;

Keywords

Palmyrah tree,
Palm sap,
Yeast,
Ethanol,
Biofuel.

ABSTRACT

The palm sap of Palmyrah tree (*Borassus flabellifer*) can be used as a source to produce bioethanol. The Palmyrah trees are distributed naturally in tropical and subtropical regions. These trees can be grown on very arid areas. The most important product of the Palmyrah tree is the palm sap. This sap is collected by the processes of tapping. The palm sap is a cheap renewable agricultural resource, which can be used for ethanol production. The potential of using the Palmyrah tree grown in India for producing bioethanol was evaluated. Tube method of extraction was introduced to extract palm sap and the yield was compared with the traditional method of extraction. The tube method of extraction gives 30% greater yield. Production of Bioethanol at the laboratory scale from palm sap was studied. The fresh palm sap fermented by air born microorganism yielded 3.5% (v/v) ethanol. The yield of ethanol has enhanced when the palm sap was fermented with the yeast strain MTCC171 with the maximum yield of 7% (v/v). Further the dilution of palm sap yielded a maximum of 12% (v/v) ethanol. The ethanol extracted from palm sap can be used as biofuel.

Introduction

There is a worldwide search for alternative fuels or energy production from renewable sources. Great attention is focused on bioethanol from renewable sources in order to use it as biofuel. Due to rapid depletion of world petroleum reserves and its rising prices, new sources of hydrocarbons must be found to supply our chemical and energy needs (Sitton et al., 1980; Lee et al., 1983). The alcohol production by fermentation offers promising alternative, as it can be produced from various renewable natural resources. In view of increasing importance of alcohol, as an alternative source for liquid fuel, a great deal of research interest in ethanol fermentation has been generated in the last three decades (Vega et al., 1987). The overwhelming advantage of fermentation is that the raw materials used in the fermenting processes are renewable (Pramanik 2003).

Because of the increasing demand for fuel ethanol, there is a need to search for high yielding processes and easily accessible technology for the production of ethanol at a reduced cost (Sree et al., 2000). Production of ethanol from biomass is one way to reduce both the consumption of crude oil and environmental pollution (Lang et al., 2001). Brazil is the pioneer in large scale motor fuel ethanol production through fermentation of sugarcane by yeast. The production of bioethanol by Brazil is about 14.2 billion liters (Licht 2005). The bioethanol is produced using the sugars present in cane syrup (Monte Algre et al., 2003).

In the present investigation steps were undertaken to produce ethanol from the already existing palmyrah tree (*Borassus flabellifer*) a renewable natural resource. The palmyrah tree is grown in coastal India and holds a prominent importance in Indian economy (Anon 1988). The Palmyrah tree is the state tree of Tamilnadu. According to the census taken in India in 1995 about 8.59 crores Palmyrah trees are present in India and out of which 5.19 crores of Palmyrah trees are present in Tamilnadu (Tamilnadu palm products 2003). This tree may grow up to 30m high and the trunk may have circumference of 1.7m at the base (Mortan 1988). This tree is a source of material for producing a variety of product such as palm sap, palm gur, palm candy. The most important product is the sap or juice. The palm sap showed high nutritive value (Debmalya Barh 2008). Khieu Borin 1996 reported that the palm sap can be collected for 3 months from male tree and 5-6 months from female tree. In India the palm sap season begins in January when the palmyrah trees start to bud and it gives off sap till the end of june. The palm sap (sugary solution) can be obtained by the excision of the meristem. Basically, starch reserves from the trunk are converted to sugar and are transported upward towards the stem apex (Fox 1977). Pethigoda (1978) describes the upward stream as a watery liquid containing dissolved salts absorbed from the soil, and the downward stream as a comparatively rich mixture of food (principally sugars) manufactured in the leaves. The sap flow is intercepted by injuring fibro-vascular tissues in the apex of the inflorescence. We use generally the young growing sites (meristematic tissues) to freshen the wounds. Tapping of inflorescence is practiced twice a day. The Palmyrah tree yields an average of 6-10 liters of sap per tree in a day. This depends upon the genetic (Kovoor 1983) and environmental factors. It is likely that the yield improvement research will produce varieties that will yield more than 100 litres of sap per palm and more than 14,800 litres of sap per hectare (Udom 1987). The palm sap is a highly nutritive drink consumed widely by Indian people; however its production has declined due to poor traditional technologies adopted for climbing the palmyrah tree and lack of maintaining the hygiene in tapping palm sap. A need for an improvement in the traditional technologies can be helpful in the palm sap production with improved hygiene. Fresh palm sap is rich in sugars such as sucrose, maltose, glucose, fructose

Tele: 914622336768
E-mail addresses: veluraja@msuniv.ac.in

and raffinose (Okafor 1978). Fermentation of the sugars and other nutrients by the endogenous yeast leads to alcohol and organic acid production. In industries the brewer's yeast *Saccharomyces cerevisae* is used for fermentation (Zaldivar et al., 2001).

In the present investigation technological processes were introduced to increase the yield of hygienic palm sap and various experimental trials were carried out to achieve maximum yield of ethanol from the extracted palm sap.

Materials and Methods

Traditional Method of Extraction

The traditional procedure adopted in the palm sap production and collection is described here. About 20 year old palmyrah trees (matured palmyrah tree) are selected and the inflorescence is crushed between two rods. Rods of different shapes are used for crushing male and female inflorescence (Figure1).

Figure 1. Rods of different shapes used for crushing male and female inflorescences

The crushing is done twice a day and for a period of one week. At the end of crushing, the inflorescences are sliced vertically (thin section) and this is being done in the morning and evening and is repeated every day. In order to carry out this work, a person has to climb twice a day physically to reach the top of the tree. A mud pot with the inner side coated with lime is tied to the inflorescence to collect the oozing out sap from the inflorescence. The traditional method of extraction of palm sap is shown in Figure 2.

Figure 2. Traditional Method of Palm sap Extraction.

Every day the tree climber goes to the top of the tree and collects the palm sap in a separate vessel. While climbing down the tree he has to carry the whole container which gives him hardship. In this method the inflorescence is exposed to air. This leads to drying up of the inflorescence and decreasing the oozing rate. The collected palm sap is contaminated with ants and dust because of the collecting vessel exposed to air atmosphere.

Tube Technology of Palm sap extraction

The man power in tapping palm sap has gone down due to hardship in climbing up the tree twice a day for processing and collection. So it is time to develop new technology to extract palm sap in an efficient and scientific way. In order to do so a new technology is introduced by us. In this new technology a tube of diameter 1 cm attached with funnels is used. A close up view of this arrangement is shown in the Figure 3.

Figure 3. New method of palm sap extraction (Tube Technology).

The inflorescences are inserted into the funnels and the tubes coming out of the funnels are linked to a single tube and this tube is inserted into the collecting vessel which is kept at the bottom of the palmyrah tree. A full view of tube technology set up developed by us is shown in Figure 4.

.

Figure 4. Tube Technology of extraction (Full view)

Care is taken to have airtight arrangement between the inflorescences and at the collecting pot in order to maintain humidity in the inflorescences. This avoids the inflorescences reaching a dry state and thus maintaining a continuous flow of sap.

Organism and Culture Media

The yeast *Saccharomyces cerevisiae*, strain MTCC171 (NCIM3090, NRRL567, ATCC9763) is a distillery strain, was provided by School of Biotechnology, Madurai Kamaraj University, TamilNadu, India. The culture was routinely maintained on YEPD slants and subcultured. The YEPD medium contained yeast extract 10.0 g, Peptone (M) – 15.0 g, d H_2 O – 950 ml, 40% solution of Glucose stock was prepared, autoclaved separately and added to the above medium to 2% final concentration. This was used for inoculum preparations and palm sap fermentation.

Distillation of ethanol

The ethanol extraction is carried out by distillation. In this a fractionating column is inserted in between the distillation flask and the distillation head. The fractionating column provides a large surface area in which the mixture can be continuously vaporized and condensed. The heating mantle is used to heat the solution taken in the conical flask. Since the boiling point of ethanol is 78.3°C. The distillation is carried out between temperatures 78°C and 80°C

Fermentation of Fresh Palm sap using cultured yeast strain

Cultures were grown in YEPD medium in a shaker for 24hrs at 30°C at 180rpm. Based on the OD measurement at 600nm indicates that 1ml of culture contains. $1X10^7$ cells/ml. 50 ml of the culture was transferred to 250 ml of palm sap (fermentation medium) in a 500 ml conical flask. This was left closed for 48hrs at room temperature. The fermented solution was distilled using a distillation set up. The procedure was repeated for various concentration ratios of culture and palm sap.

Results and Discussion

Traditional Method and Tube method of extractions – A Comparative study

In order to investigate and compare the traditional method and tube method of palm sap extraction, we have collected the palm sap from an inflorescence of a single male palmyrah tree during the month of March from 7.30 am to 5.30 pm at the intervals of 30 minutes. The yield is measured. This is done for four days randomly for the traditional method and the tube method. The average yield is taken for comparison and is shown in Figure 5, (standard errors are indicated as error bars). It is clear from the Figure 5 an increase in yield of palm sap was obtained in the tube method of extraction as compared with traditional method of extraction.

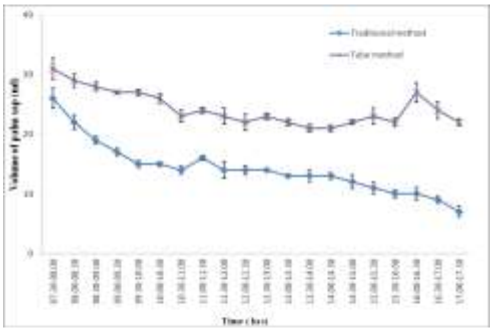

Figure 5. Average volume of palm sap collection over 30 minutes period per inflorescence by Traditional and Tube methods

In order to calculate the percentage of increase in palm sap production the following formula is used.

$$\text{Percentage of increase} = \frac{(\text{Amount of palm sap from Tube method} - \text{Traditional method})}{\text{Amount of palm sap from Tube method}} \times 100$$

The tube method of extraction recorded a yield of 30% increase in palm sap when compared with traditional method. In this method of extraction, a good quality, pure palm sap is ensured because of the closed set- up. Ants, bees and dust particles will not settle in palm sap in the tube method of extraction whereas the traditional method of extraction is prone to these contaminants. In traditional method the tappers tap the inflorescences twice a day and they need an assistant at the base of the trunk to receive the collected palm sap from the top of the tree. But in the tube method of extraction the palm sap can be collected from the bottom of the tree directly. In the tube method of extraction, the airtight arrangement between the inflorescence and the collecting vessel helps in avoiding the inflorescence go dry. This may help the climber to go to the top of the tree to tap the inflorescence once a day instead of twice a day (in traditional method), thus decreasing his work load up to 50%.

Ethanol extraction from palm sap

The fresh palm sap is distilled at 78°C to 80°C using the distillation set up and the yield is only 3.5% (v/v). The % of ethanol extracted is calculated from the formula

$$\% \text{ of ethanol extraction} = \frac{\text{Amount of distillate collected}}{\text{Amount of palm sap}} \times 100$$

Nuclear magnetic resonance spectrum is recorded for fresh palm sap and the distilled product. The spectrum is shown in Figure 6 and 7. Dramatic difference is noticed from the spectrum recorded for palm sap and the distilled product. The spectrum of the distilled product shows a singlet, triplet and a quartet, the chemical shift values matches with the chemical shift values of ethanol. This confirms that the distilled product is ethanol.

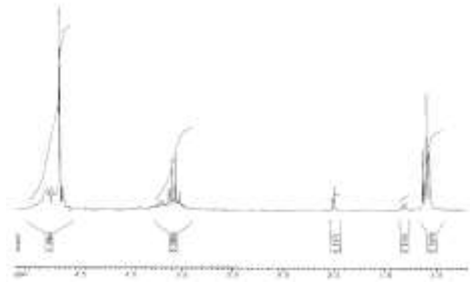

Figure 6. H^1 N. M. R Spectrum of Palm sap

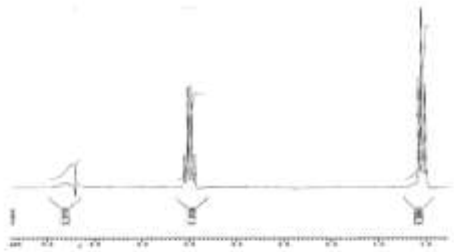

Figure 7. H^1 N. M. R Spectrum of Palm sap distilled product.

This fermentation is due to the air born microorganisms and the fermentation is not enhanced even the palm sap is left for longer duration (30 days). These microorganisms cannot survive in high alcohol content and hence no further increase in ethanol content is noticed. To enhance the fermentation the cultured yeast strain MTCC171 was used. This cultured yeast strain can survive at high alcoholic content so its activity does not stop when the alcoholic content increases. Here, 50ml of yeast culture (MTCC171) was added to 250 ml of palm sap. The solution is left without disturbing for 48 hours. This yielded about 7% (v/v) of ethanol. It is obvious that the ethanol extraction has almost doubled due to the yeast strain MTCC171. There is no appreciable change in ethanol extraction is noticed even when the solution is left for long hours (more than 48 hours). To increase the growth of yeast, dilution procedure is carried out. In this procedure the solution which yielded 7% (v/v) ethanol is diluted using distilled water to 100% and the solution is allowed to ferment for 48 hours. This has enhanced the ethanol extraction to 12% (v/v). This is the maximum yield of ethanol we have achieved from palm sap using the yeast strain and dilution. An optimum period of 48hrs is needed for fermentation to achieve the above yield.

Conclusion

The ethanol extracted from palm sap can be blended with petrol and can be used as biofuel. This can reduce the consumption of petroleum fuels as well as air pollution. Since ethanol is produced from already existing palmyrah tree hence it is a renewable resource for biofuel. Utilizing these trees for the production of biofuel will also contribute to enhance rural economy.

Acknowledgement

S.Arunavathi acknowledges the Manonmaniam Sundaranar University for the University Stipendiary Research Fellowship.

References

Anon. The wealth of India: Raw materials. Coun.Sci. Indus.Res. New Delhi. 1988.

Debmalya Barh, Mazumdar BC. Comparative Nutritive Values of Palm saps Before and after their partial fermentation and effective use of wild date (Phoenix sylvestris Roxb.) sap in Treatment of Anemia. Research journal of Medicine and Medical Sciences. 2008; 3 (2): 173-176.

Fox JF. Harvest of the Palm. Ecological Change in Eastern Indonesia. Harvard University Press, Cambridge, Massachusetts, and London, England 1977, 290.

Khieu Borin B. A study on the use of the sugar palm tree (*Borassus flabellifer*) for different purposes in Cambodia. M.Sc. Thesis Swedish University of Agricultural Sciences, Uppsala, 1996, 67.

Kovoor A. The Palmyrah Palm: Potential and Perspectives. FAO Plant Production and Protection. FAO, Rome, 1983; 52, 77.

Lang X, Macdonald DG, Hill GA. Recycle Bioreactor for Bioethanol Production from Wheat starch II. Fermentation and Economics Energy Sources. 2001; 23, 427- 436.

Lee JM, Pollard, JF Coulman GA. Ethanol Fermentation with Cell Recycling: Computer Simulation. Biotechnol Bioeng. 1983; 22, 25497 - 25511.

Licht FO. World ethanol and biofuels Report, 2005; 3, 15.

Monte Algre R., Rigo M, Joeker L. Ethanol fermentation of a diluted molasses medium by Saccharomyces cerevisiae immobilized on Chrysotile. Braz Arch. Biol.Technol. 2003; 46, 751-757.

Mortan J F. Notes on distribution, propagation and Products of Borassus Palms (*Arecaceae*). Economic Botany, 1988; 42 (3), 420-441.

Okafor N. Microbiology and Biochemistry of palm wine, Advances in Applied Microbiology. 1978; 24, 237-254.

Pramanik K. Parametric studies on Batch Alcohol Fermentation using Saccharomyces Yeast extracted from Toddy. J. Chin. Inst. chem Engrs. 2003; 34 (4): 487-492.

Sree NK, Sridar M, Suresh K, Banet IM, Rao LV. High alcohol production by repeated batch fermentation using an immobilized osmotolerant Sacch.cerevisia. J. Ind. Microbial. 2000; 24, 222-226.

Sitton OC, Gaddy JL. Ethanol Production in an Immobilized Cell Reactor. Biotechnol Bioeng. 1980 22; 1735-1748.

Tamilnadu Palm Products Development Board. Policy note for 2002-2003.

Pethiyagoda U. Coconut Inflorescence Sap. Perak Planters Association Journal. Malayasia, 1978; 86-91.

Udom DS. Economic of oil palm tapping, Nigerian Journal of Palms and oil Seeds, 1987; VII (1): 56-77.

Vega JL, Clausen EC, Gaddy JL. Acetate Addition to an Immobilized Yeast Column for Ethanol Production. Biotechnol. Bioeng. 1987; 29, 429- 431.

Zaldivar J, Nielson J, Olsson L. Fuel ethanol production from lignocellulose a challenge for metabolic engineering and process integration. Appl. Microbiol. Biotechnol. 2001.