Advanced Journal of Microbiology Research Vol. 2017
Available online at http://internationalscholarsjournals.org/journal/ajmr

Short Communication

Production of bio-ethanol from *Pectobacterium carotovorum* induced soft rotten potatoes

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Accepted 17 May, 2016

The potato is a tuberous crop that adapt readily to diverse climates. The potato contains vitamins and minerals that have been identified as vital to human nutrition as well as an assortment of phytochemicals, such as carotenoids and polyphenols. *Pectobacterium carotovorum* is the causative agent for the soft rot induced in potatoes across temperate and tropical regions. Production of ethanol from 10 potato cultivars which were rotten by soft rot (*P. carotovorum* induced) was effected. Ethanol yield of about 6 - 10 ml / 100 gm of potatoes was achieved. The effect of pH in the yield of ethanol was also determined in the ten cultivars by varying the levels of pH across the spectrum and it was found that pH had a considerable impact on bio-ethanol production. This method of ethanol production is easy and environmental friendly in nature.

Key words: *Pectobacterium carotovorum,* phytochemicals, carotenoids, polyphenols, potatoes.

INTRODUCTION

Ethanol fermented from renewable resources for fuel or fuel additives are known as bio-ethanol. Additionally the ethanol from biomass- based materials is considered as bio-ethanol (Grassi et al., 1999). Currently there is a growing interest for ecological sustainable bio-fuels. In many parts of the world bio-ethanol is already used as additive in some gasoline products instead of toxic MTBE and TAMES (EC report, 2000).

Bio-ethanol production from potatoes is based on the utilization of rotten potatoes. Rotten potatoes are obtained from 5 - 20% of crops as by-products in potato cultivation (Kimmo et al., 1999). India produces 1.5 million kilograms of waste and rotten potatoes per year. Because this rotten potato based bio-ethanol production is being less focused, there is a strong need for its research and development. Therefore, the aim of the study was to develop different analytical methods for bio-ethanol production from *Pectobacterium carotovorum* induced soft rotten potatoes and to study the effect of potato cultivar on bio-ethanol.

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P. carotovorum is a rod shaped bacterium originally isolated from carrot, is a plant pathogen and opportunistic human pathogen, causative agent of soft rot and blackleg potato (*Pectobacterium atrosepticum*) diseases (Abouzied et al., 1983; Jarl et al., 1969) . Symptoms of soft rot include rotted tissues that are wet, cream to tan in colour and soft. Rot begins on the tuber surface and progresses inward. Infected tissues are sharply delineated from healthy tissue by dark brown or black margins. Shallow necrotic spots on the tubers result from infections through lenticels (Dubios et al., 1953). Rotting tissue is usually odourless in the early stages of decay, but develops a foul odour as secondary organisms invade infected tissue. Soft rot can also infect wounded stems and roots (Forney et al., 1977).

MATERIALS AND METHODS

High quality potato that has been half rotten was taken for the process to produce ethanol. 100 g of rotten potato was weighed exactly from each of the ten cultivars. It was then cut into small pieces for enhancing the mashing process. It was grinded to an optimum size using a mixer. Later the mash was heated in hot air oven for about seven hours at 70°C. It was later heated in a microwave oven for 20 min till all the water content in it were dried

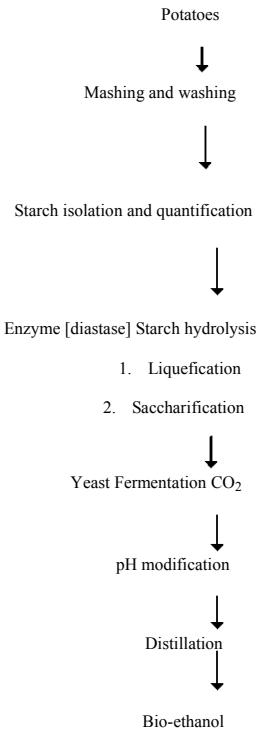

Figure 1. Flow chart for the production of bio-ethanol from rotten potatoes fermented by *Pectobacterium carotovorum*

Figure 2. Conical flasks containing crude bioethanol.

up. Now a solid mass was obtained. This mass was sliced into 5 mm [maximum size]. 500 ml of water and 3 g of enzyme diastase was added to it and was boiled for about half an hour at 30°C. After the saccharification, enzyme diastase was again added and cooled down at room temperature for 60 min. Now 3 g of yeast was added the content was adjusted to varied pH in separate conical flasks with the addition of phosphoric acid and enzyme diastase (Keer et al., 1950; Verlag et al., 1984) (Figure 1).

pH study and ethanol detection

The pH of the biomass was made acidic with addition of phosphoric acid and was made basic with the addition of sodium hydroxide

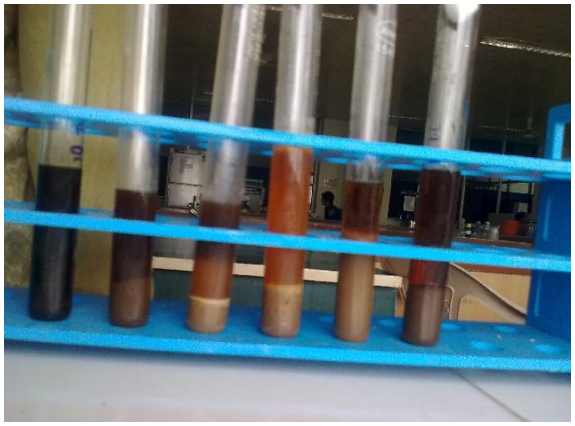

Figure 3. Bioethanol production at difference pH.

Figure 3). Presence of ethanol was detected by simple lab method (– the Iodoform test (Kosaric et al., 1983).

Iodoform test

The flask containing the solution of different pH was collected separately in test tubes for testing the presence of ethanol. 10 drops of solution water was taken in a test tube and 25 drops of iodine was added along with 10 drops of NaOH in the test tube. After few minute cloudy formations in the test tube gives the conformation for the presence of ethanol, it also gives yellow precipitate and an antiseptic smell (Figure 4). Iodoform test was performed for various pH levels and the colour intensity was observed.

RESULTS AND DISCUSSIONS

Dry matter contents of potatoes

Dry matter contents varied from 16.0 to 27.3%. Dry matter contents of the potatoes were observed to have an effect on starch hydrolysis potatoes with high dry matter content were difficult to process.

Ethanol yield

Alcohol yields varied significantly. The highest yield was obtained when the potato solution slightly acidic (pH 4 and 5) and the alcohol yield was almost nil when it was basic and at high pH. The yield was around 7 - 8 ml / 100 gm of rotten potatoes (Figure 2).

The dry matter content was found to be in the range of 13 - 25% with starch content in the range of 8 - 19%. The highest ethanol yield was found to be 10 ml/100 g of potatoes soft rotten by *P. carotovorum.* The overall difference in the yield of bioethanol in comparison with the theoretical yield was found to be in the range of 106 - 146% (Table 1).

pH difference also had the difference in the ethanol yield from various cultivars. The colour intensity was broadly classified as pale or dark depending on the input

Table 1. Properties of potato cultivars.

Cultivar	Dry matter content (%)	Starch content (%)	Ethanol yield (EtOH/100 g)	Theoretical yield (EtOH/100 g)	Yield (%)
1	18.4	13.2	8.3	7.8	106
2	16.1	10.6	7.1	6.5	109
3	17.6	14.5	9.6	7.2	133
4	15.5	-	6.5	-	-
5	13.2	8.9	7.2	5.9	122
6	17.8	14.3	8.9	7.7	116
7	24.5	19.2	8.0	5.5	146
8	22.2	-	7.4	-	-
9	20.3	17.1	10.0	8.3	121
10	16.7	14.6	7.8	5.9	132

Table 2. Iodoform test results.

pH	Enzyme diastase added (g)	Colour obtained after the iodoform test
0.8	2	Pale yellow
1.1	2	Pale yellow
4	2	Dark yellow
5	2	Dark yellow
7	2	Pale yellow
11	2	Pale yellow

from spectrophotometer. The amount of diastase enzyme added was kept constant throughout, 2 g (Table 2).

Conclusion

The production of bio- ethanol from rotten potatoes seems very much possible and the yield was also quite encouraging. The 2 main parameters we had to quantitatively determine were the relationship between the amount of diastase enzyme added and the quantity of bio-ethanol produced. The yield of 7 - 8 ml / 100 gm was achieved at the addition of 2 g of diastase enzyme / 100 g of rotten potatoes should be economically viable considering the market cost of ethanol. The pH at which maximum bio-ethanol production was there was the 2nd point to be established and optimum pH was found to be 4 - 5. This user friendly method can be used to easily produce ethanol when it is required with minimum requirements and an economical cost. This method should be a boon for lower and middle class students.

ACKNOWLEDGEMENTS

We would like to thank the management of SRM University, Chennai for providing the necessary infrastructure to carry out this work. Heartfelt gratitude also goes to our family and all friends who stood by us.

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