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Production of gluconic acid by using some irradiated microorganisms

Ashraf S. Ahmed[*], *Souzy S. Farag*, *Ismail A. Hassan*, *Hany W. Botros*

Plant Research Department, Nuclear Research Center, Atomic Energy Authority, Egypt

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ABSTRACT

The objective of this study was to isolate the potential fungal isolates have the ability for gluconic acid production by using some agro industrial byproducts as sugarcane molasses, banana-must and grape-must. The effect of gamma-irradiation on the most potent isolates and the fermentation conditions as pH, incubation temperature and incubation period was also investigated. Results showed that the most potential fungal isolates were *Aspergillus niger*, *Penicillium puberulum* and *Penicillium frequentans* whereas their gluconic acid production was 62.17, 56.25 and 39.69 g/L, respectively on Czapek's Dox media at 28 ± 1 °C, pH 6 for 7 days fermentation period. Irradiation of the three most potential isolates at 0.1, 0.2, 0.3, 0.4 and 0.5 kGy doses of gamma ray showed that 0.1 kGy dose caused an increase in gluconic acid production whereas it was 69.35, 60.17 and 40.31 g/L by the three potential isolates respectively. Data showed that utilization of sugarcane molasses, banana-must and grape-must as a sole carbon source in gluconic acid production by the three potential (0.1 kGy) irradiated isolates at pH 6, 30 °C for a 7 days incubation period caused increasing in gluconic acid production whereas the productivity of the three (0.1 kGy) irradiated isolates (*A. niger*, *P. puberulum* and *P. frequentans*) was 69.87, 63.14 and 51.28 g/L by utilizing sugarcane molasses, 61.28, 56.37, 47.15 g/L by utilizing banana-must and 54.25, 52.75 and 44.75 g/L by utilizing grape-must.

1. Introduction

Gluconic acid is a mild organic acid that has gained much interest as it has many industrial applications such as in the pharmaceutical, food, animal feed, textile and leather industry (Singh, Pereira, & Singh, 1999). It is also applied as additive in cement to control the setting time and increase strength and water resistance.

Gluconic acid can have further applications for the solubilization of phosphate (Fenice, Selbman, Federici, & Vassilev, 2000; Rodriguez, Conzalez, Goire, & Bashan, 2004; Vassilev, Vassileva, Fenice, & Federici, 2001) and as cement additive (Hustede, Haberstroh, & Schinzig, 1989; Singh, 1976). Gluconic acid is a noncorrosive, nonvolatile, nontoxic mild organic acid so it imports a refreshing sour taste in many food items. In the European Parliament and Council Directive No. 95/2/EC gluconic is listed as a generally permitted food additive (E574).

* *Corresponding author*.
E-mail address: ashrafsa2000@yahoo.com (A.S. Ahmed).
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The US-FDA (Food and Drug Administration) has assigned sodium gluconate a GRAS (generally recognized as safe).

The overall demand of this organic acid has been increased for almost 20 years and recently production is amounting to more than 60,000 tons per year and still growing (El-Enshasy, 2003; Singh et al., 1999). Commercially, gluconic acid is produced by three different methods; chemical oxidation of glucose with a hypochlorite solution (Kundu & Das, 1984), electrolytic oxidation of glucose solution containing a known value of bromide (Amberkar, Thadani, & Doctor, 1965), or fermentation process where specific microorganisms are grown in medium containing glucose and other ingredients (Hill & Robinson, 1988; Lee, Pan, & Lebeault, 1998; Shah & Kothri, 1993). The microbial fermentation processes offer attractive techniques for the gluconic acid production to alleviate the problems related to chemical production such as the inevitable side reactions and also to further economize the bioprocess (Singh et al., 1999; Velizarov & Bechkov, 1994).

A wide group of microorganisms particularly filamentous fungi have the ability for gluconic acid production (Cochrane, 1958; Lockwood, 1975). The production of gluconic acid is mainly done in batch cultivation using several species belonging to the following fungal genera, *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Gliocladium* (Lockwood, 1975; Petriuccioli, Piocioni, Fenice, & Federici, 1994; Rosenberg, Svitel, Rosenbergova, & Sturdik, 1992; Singh, Sharma, & Singh, 2001a). Among the different fungal genera, it has been reported that the accumulation of large amounts of the gluconic acid and its salts are restricted to certain species of *Aspergillus* especially *Aspergillus niger* which considered as the most industrially important gluconic acid producer in fermentation industry (El-Enshasy, 2003; Roukas, 2000; Sankpal, Joshi, Sutar, & Kulkarni, 1999; Sankpal, Cheema, Jambe, & Julkami, 2001).

A large quantity of raw fruit materials during storage undergo decomposition and generate a waste that may cause environmental pollution. Utilization of these waste materials can be a part of environmental pollution control on one hand and production of value added products of commercial significance on the other, thus changing their status from waste to potential provider.

Agro-food byproducts such as grape-must, banana-must and sugarcane molasses contain high concentrations of sugars and can be considered as potential substrates that are easily available and economical waste carbohydrate sources for gluconic acid production by different fungal species. Gamma-irradiation affects the activity of some fungal species during fermentation processes. Chakravarty and Sen (2001) showed that low dose of ionizing radiation on microorganisms is responsible for accelerated enzyme activity.

Gherbawy (1998) showed that the lowest dose of gamma irradiation (1 MilliCurie for 10 min) enhanced three isolates of *A. niger*, investigated to produce more biomass and polygalactronase, pectinmethylglacturonase, cellulase and protease. Haggag and Mohamed (2002) indicated that *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma koningii* irradiated with 0.5 kGy dosage resulted in the highest percentage of pathogen growth reduction by producing highly active exoenzymes.

Afify, Abo El-Seoud, Ibrahim, and Bassam (2013) indicated that the biomass of *Trichoderma* spp. was increased and reached its maximum at 250 Gy and as a general trends, the gamma radiation over than 0.25 KGy reduce the growth of *Trichoderma* spp.

The present study is aimed at evaluating some economical wastes as grape-must, banana-must and sugarcane molasses as a sole source of carbon in the fermentation process by using some gamma-irradiated fungal species for the gluconic acid production.

2. Materials and methods

2.1. Isolation and identification of organisms

Different fungal isolates were obtained from cultivated soil samples and waste materials of sugarcane processing from Hawamedia Distilleries Factories. Also some other organisms were isolated from wastes of the grape-must and banana-must collected from the fruit local market in 6 October City, Cairo. The dilution plate method described by Johnson, Curt, Bond, and Fribourgy (1959) and Czapek's Doxs Agar medium (Oxoid Limited, 1982) supplemented with rose Bengal (1/15,000, W/V) as bacteriostatic agents (Smith & Dawson, 1994) was used for isolation of fungi. For the isolation, plates were incubated at 28 ± 2 °C for 7 days and developing fungi were purified and identified by macro and microscopic characteristics using the following references (Barron, 1998; Carmichael, Brycekendrick, Conners, & Sigler, 1980; Domsch, Gams, & Anderson, 1980; Gilman, 1957; Nelson, Taussow, & Marasas, 1983; Paper & Fennell, 1977). Isolated fungi were maintained on potato dextrose agar (PDA) slants and incubated at 30 °C for 7 days. The slants were stored at 4 °C and sub-cultured every month. The spore suspension was prepared by suspending the spores on the slant in 10 mL of sterilized saline solution.

2.2. Fermentation technique

Gluconic acid fermentation was carried out by submerged fermentation in 250 mL cotton wool plugged Erlenmeyer flasks with 50 mL of fermentation media of Czapek's Dox Broth consisted of (g/L) sucrose 30.0, $NaNO_3$ 3.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, KCl 0.5 and $FeSO_4 \cdot 7H_2O$ 0.01 having pH 6.0. The medium was modified by substituting sucrose with 120 g/L glucose from each previously diluted substrate type, i.e. grape-must, banana-must and crude molasses.

2.3. Preparation and purification of grape-must

Market-refused red grapes (100% ripened) that did not meet with the quality norms were used in fermentation reaction for gluconic acid production. Clarification of grape-must was followed as described by Grassim and Fauquembergue (1996) with slight modifications. Briefly decomposed and market-refused grapes were collected (1 kg) and mixed with eleven double, distilled water.

These were then steamed, crushed and heated at 80 °C for 30 min for release the red color from the grape skin and to