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ORIGINAL ARTICLE

Using the Rice Husk Filtrate as Alternative Medium for Oxalic Acid Production Byaspergillus Niger

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ABSTRACT

The aim of this study was to select cheaper and high yielding alternative medium, like rice husk filtrate, for oxalic acid production by A.niger fungus, in comparison to other culture media that have been used including, Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), and Sabouraud Dextrose Broth (SDB). The results showed that the highest percentage of oxalic acid production happens with rice husk filtrate medium, when its productivity was (12.02%), and reached (15.56 %), when rice husk filtrate was used as a solvent for (SDB) powder. It was followed by (SDB) medium, with a production rate of (5.06), then (SDA) with a production rate of (4.94), and (PDA) which gave the lowest production rate (3.67%)

Keywords: Oxalic acid, A.niger, Rice Husk Filtrate medium

INTRODUCTION

Oxalic acid (OA): is an important organic acids that are used in medical, industrial, and biological fields (Gilberto et al ., 2020). There are several methods for OA production, since the researcher Berthelote obtained oxalic acid chemically through oxidation of acetylene, propylene, ethylene, and allylene by potassium permanganate (Felter & Lioyd ,1898 ; Foster, 1952), as thesemethods are expensive and dangerous, so recently it is turned toward the biological methods and through exploiting the microbial fermentation.

Different types of bacteria, such as Pseudomonas fluorescens were used in OA production (Hamel et al ., 1999), other reseachers used fungi, such as certain types of Zygomycota , Basidiomycota, and Ascomycota, as well as some lichens (Dutton & Evan , 1996), It is very interesting to produce the organic acids by cultivating the microorganisms on cheap raw materials, and to achieve the highest productivity, also it become possible to utilize a sugar-rich industrialwastes in organic acid production (Ali& Zulkali , 2011).

The researchers were used a different media and food materials for fungal cultivation to obtain the best productivity, taking in to account source availability, cost, and the fungal isolate. The molasses was used in several concentrations by Al-Mehana et al .,(2021) as a culture medium for A.nigerandA.flavus, and the best concentration was 5.6, which gave a productivity of 29.5g/L with A.niger and 26 g/L with A.flavus. Other researchers Al-Ajili (2005) were used the date juice, and the date residuesinfusion media, where the result showed that date residues infusion had productivity of 15.76 g/L, also it proved that no difference present between date juice and date residues infusion, in OA production capacity. Several culture media (CDA, PDA, OA, SDA) were used in the study Al-Jubouri & Ban (2013) to compare their effect on OA production, (SDA) gave the higher amount of OA, followed by (CDA), (OA), and (PDA) respectively. Whey was used Bohlmann et al .,(1998) to cultivate A.niger for OA production, it is confirmed that OA was obtained in large quantities when phosphorous supplemented whey was used Cameselle et al .,(1998), but it is also confirmed that whey contains a sufficient amount of nutrients for A.niger to grow and produce OA Santoro et al .,(1999). The solid-state fermentation technique was used to cultivate fifteen strains of brown-mold on sawdust, after treating it with copper citrate, as (700 mM) of OA have been obtained after four weeks of incubation, by a strain belonging to the genus Antrodia(Green & Clausen , 2003). The genus A.niger was cultivated on sawdust medium treated with arsenic, copper, and chromate at pH 6 by Kartal et al .,(2004), as 13.4 g/L of OA was obtained, and about 97% arsenic, 49% copper, and 55% chromate were extracted, after 10 days of incubation. Another researcher made a comparison between different cultures media including sucrose mineral salts medium, molasses medium, and brewery medium, it was found that sucrose mineral salts is the best medium for OA production (Mulligan 2003). The researchers Podgorski & Lesniak (2003) concluded that cane molasses is not suitable for OA production. Fats also were used as a medium for OA production, when a mutant isolate of Aspergillus niger was cultivated on 50 g fat, and 6 g OA was obtained after 7 days of incubation (Rymowicz & Lenart , 2003).

The recent studies tended to use cellulosic and lignocellulosic agricultural wastes, such as (rice straw, wheat straw, corn stalks, and bagasse), as well as solid waste from the paper industry and food industries waste. The studies also directed to find the best conditions with a lowest cost, and our study came to choose an alternative medium that is cheap and available like rice husk filtrate, (a by-product of the rice industry). The current study included the following axes:

1 Isolation, purification and identification of Aspergillus niger from the soil.

2 chose an alternative cheap medium, and estimate the amount of OA produced from it.

MATERIALS AND METHODS

Isolation & diagnosis of Aspergillus niger: One kilogram of soil was taken for each sample, and from different gardens, placed in clean tightly closed nylon bags, and transferred to the lab where the dilution method was done according to Al-Khalil (2005). Then cultured on PDA media and incubated for 7 days. Fungal isolates were purified accordingtoKown-chung & Bennett (1992),bytouchingand transferring the mixed colonies separately to new SDA media that incubated at 25 ° C for 7 days, the process was repeated until pure single colonies have been obtained. The diagnoses are based on morphological features, such as the shape, color, and growth behavior, as well as the microscopic features, by preparing methylene blue stained smears and noting the colonies' features, such as the hypha (divided or non-divided), conidial shape, and the sporangium, the diagnosis depended on the references (Afzal et al ., 2013 ; Carmen & Sciortion , 2017 ; Kidd et al ., 2016).

Culture media:

1. Potato Dextrose Agar (PDA), prepared according to Saito & Machida (1999) .
2. Sabouraud Dextrose Agar (SDA), prepared according to Ellis (1994) .
3. Sabouraud Dextrose Broth (SDB), provided by (Liofilchem/Italy)
4. Rice husk filtrate medium :

This medium was prepared in the laboratory according to the method (Ang et al ., 2009).Where a quantity of rice husks was taken from one of the grinders in the city of Al-Diwaniyah and these husks were washed with plain water first and then with distilled water for the purpose of removing impurities and dust stuck to it. The rice husks were placed in a glass beaker with a capacity of 500 ml, a quantity of distilled water was added to it, then 10 ml of nitric acid was added to it, and the volume was filled with distilled water to 500 ml, then the mixture was boiled at a

temperature of 100 ˚ C for 8 hours, then left to cool and then filtered using filter paper. Whatman No.1 type, the sediment was neglected and the filtrate was taken and sterilized with an Autoclave as mentioned in the previous paragraphs. This filtrate was used as a cheap alternative medium for the production of oxalic acid.

The media were supported with the optimal conditions (that were previously reached through the current study) and inoculated with the selected A.niger isolate, then incubated at 25 °C for 7 days. After that, the media was filtered and the filtrate was titrated with potassium permanganate to estimate the amount of OA produced.

Evaluating the percentage of OA production: The percentage of oxalic acid produced from A. niger growing on a medium (SDA, PDA) by cutting the nutritional medium growing on the fungus at the age of seven days using a sterile knife in the form of small pieces. For ten minutes, the mixture was then filtered by filter paper, then the filtrate was taken and placed in clean and sterilized flasks of 100 ml, and each beaker contains 25 ml of the filtrate. As for the medium (SDB) and the medium of the rice husks leaching and the leaching of the rice husks as a medium solvent (SDB) is placed directly in the blender, then filtered by filter paper, then 25 ml of the filter is taken and placed in a 100 ml beaker, according to (Maxwell & Lumsden , 1970). Then the percentage of oxalic acid was estimated according to the method described before (Bateman & Beer , 1965) by smearing with potassium permanganate KMnO4 (0.02 N) until the appearance of the pink color as in Figure (1) and the percentage of acid was calculated on the basis that every 1 ml of potassium permanganate (0.02 N) equivalent to 1.2653 mg of oxalic acid .

Oxalic acid (%)= potassium permanganate consumed volume x 1.2653

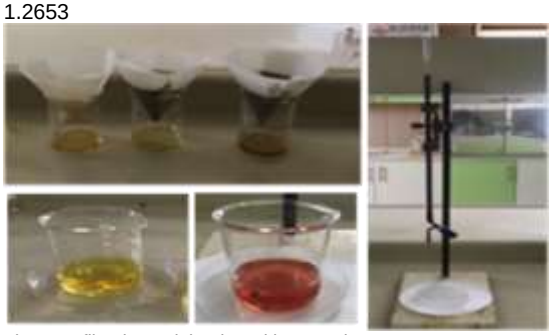

Figure 1: filtrationand titration with potassium permanganate

RESULTS AND DISCUSSION

The statistical analysis demonstrates significant differences between the productivity of the studied media, at the probability level (0.05).Table (1), and figure (2) show the OA production rates, which was (12.02%) with rice husk filtrate medium, and reached (15.56%) when rice husk filtrate was used as a solvent of (SDB) powder. Then (SDB) medium with a production rate of (5.06), followed by (SDA) medium with a production rate of (4.94), and finally, (PDA)medium which gave the lowest production rate (3.67). No significant differences were found between (SDB) and (SDA). The high productivity of (SDB and SDA) compared to (PDA) may be due to the containing of dextrose as a carbon source and peptone as a nitrogen source, this results agreed with (Mwangi et al ., 2012).

Table 1: the percentages of OAproduction according to the cultural media

The media	The percentages of OA production
)PDA(	3.67
)SDA(	4.94
)SDB(	5.06
rice husk filtrate	12.02
rice husk filtrate + SDB	15.56
$LSD_{0.05}$	0.14

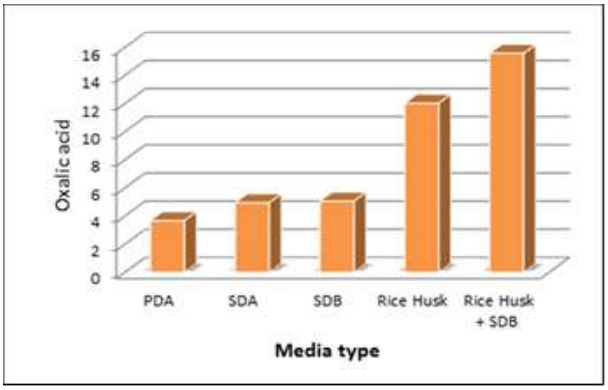

Figure 2: the effect of media type on OA production rate .

The high productivity of rice husk filtrate, may be due to having many compounds, which improve fungal growth and secondary metabolites production, rice husk filtrate consists of Cellulose (40-50%), Lignin (25-30%), salts and inorganic compounds (15-20%), and water (8-15%) (Giddel & Jivan , 2007). It has been observed that when rice husk filtrate is treated with acids such as sulfuric acid, nitric acid, and phosphoric acid, or bases such as calcium hydroxide and sodium hydroxide, this will decomposes the complex materials into simpler substances, which are consumed easily by the fungi (Ang et al ., 2009).Some studies also said that some fungi, including A.niger, can produce extracellular enzymes that breaks down the cellulose materials into simple sugars, which can exploit by the fungus for growth and reproduction (Reddy & Pushpa , 2012).

Based on the results of the current study, and due to the absence of a previous study on the production of oxalic acid using a rice husk filtrate medium, it becomes clear that rice husk filtrate could be used as a perfect medium in the production of OA, and even other organic acids .

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