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Research Article

Some Factors Affecting the Production of Itaconic Acid by *Aspergillus terreus* from Carob Pod Extract

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Abstract

The present investigation was developed to study the possibility of production the Itaconic acid from carob pod extract as a substrate by the fungus *Aspergillus terreus*. Many experiments were carried out throughout this study to determine the best cultural conditions for the high production of itaconic acid. The best itaconic acid production was achieved after 3 days of incubation at 33˚C with initial pH value of 3.5 and the percentage of yields was 756.11% (38.89g/l). According to the results of designated experiment to find out the best concentration of the nitrogen source in the form of urea that stimulated highest production of itaconic acid 541.68% (35.77g/l) was achieved at concentration of 0.3%. This results clearly suggested that the fungus *A. terreus* possess the metabolic ability to convert low grade material into high itaconic acid product.

Keywords: Itaconic Acid; Aspergillus Terreus; Carob Pod Extract; Incubation Periods; Temperatures; Nitrogen Level

Introduction

In the developing and developed countries year after year there has been an increasing use of organic acids particularly itaconic, gloconic, lactic, fumaric and kojec acids [1]. Biosynthesis of itaconic acid in *Aspergillus terreus* proceeds through formation of citric acid [2]. Itaconic acid is an unsaturated dicarboxylic acid, crystalline, relatively non-toxic with a melting point of 167 - 168˚C and density of 1.632 [3]. The property that makes itaconic acid a uniquely valuable compound is the conjunction of the two carboxyl groups and methylene group [1]. The methylene group is able to take part in addition polymerization giving rise to polymers with many free carboxyl groups that confer advantageous properties [4]. Itaconic acid is known to be produced on a commercial scale using only *Aspergillus terreus* and *Aspergillus itaconicus* [3]. In previous studies, attempts were made to develop a potent strain for improved production of itaconic acid. Alternatively, with a view to reducing the substrate costs, cheap and abundantly available substrates such as fruit wastes, which had not been reported earlier were used [1]. Moreover, starchy materials such as corn starch, soft weat flour, potato flour, cassava flour, sorghum starch, sweet potato and industrial potato flours, either acid or enzymatically hydrolysed, were also used as substrates for itaconic acid production by *Aspergillus terreus* NRRL 1960 [5]. The widespread uses of itaconic acid in polymers and in synthesizing N-substituted pyrrolidones have resulted in its increased demand [6]. Itaconic acid used as monomer or comonomer for plastics, resins, synthetic fibers and elastomers [3,7] and used as producing dental sticker(adhesive) [8]. Some other itaconic derivatives are used in medicine, cosmetics, lubricants and herbicides [8,9]. The aim of the present work was performed to evaluate the possible utilization of partially hydrolysed carob pod-based materials as substrates for high production of itaconic acid using the *A. terreus.*

Experimental Study

The Microorganism

Aspergillus terreus Thom was obtained from Egypt microbial culture collection cairo MIRCEN. During this work the culture was maintained on potato dextrose agar slants and subcultured every month.

Component of Growth Medium

Carob Pod Extract Medium: The carob pods is a fruit of carob tree (*Ceratonia siliqua*) were collected from natural willed plants in which the carob pod syrup was used as carbon source in this study. The medium containing 10% sugar was prepared as previously described by EL-Budony [10]. Different concentrations of urea % (0.00, 0.20, 0.30, 0.40, 0.50, 0.60 and 0.70) as a nitrogen source were used. The hydrogen ion concentration of the medium was adjusted at 3.5.

Cultural condition

The fermentation media were adjusted to pH 3.5. After that, the media were distributed into 250 ml conical flasks in triplicate samples receiving each 50ml of broth medium, thereafter plugged and autoclaved for 15 minutes at 121˚C, after cooling the culture flask were incubated with 2% of fungal spore suspension. The inoculation culture flasks were incubated for sufficient time at sufficient temperature value and at specifically interval three replicates of each treatment were withdrawn for further analysis.

Analysis methods

Chemical content of hydrolysis of carob pod peels (total protein, fat and ash percentages) was determined according to AOAC [11,12]. The initial and final pH of the culture media were adjusted by 1N HCl or 1N NaOH using hydrogen ion concentration instrument pH meter. Biomass of fungal dry weight was determined according to EL-Budony [10]. Initial and residual sugar estimated using the method of Dubois, Gilles, Hamilton and Smith (1956). Itaconic acid content was determined according to Friedkin [13].

Results and Discussion

1-Chemical content of hydrolysis of carob pod peels was given in table 1.

Chemical content	Sugar	Protein	Lipid	Ash
Percentage %	50	2.92	1.4	2.92

Table 1: The percentages of Chemical content of hydrolysis of carob pod peels.

2-Effect of Different Incubation Periods on The Production of Itaconic Acid by The Fungus *A. terreus*

The effects of different incubation periods on the production of itaconic acid have been studied and the results were given in table (2). As shown, the amount of itaconic acid increased with the incubation time reaching 35.75g/l (451.41%) after three days of incubation and then decreased again with the time reaching 5.59g/l (64.16%) after six days. It is also clear that most of the carbon source present in the fermentation media was utilized by the fungus during the incubation periods. Present variance in the final PH values since, declined in some values and increased in other values. The results also showed that, the present of direct relationship between the fungal biomass dry weight and periods of incubation, and the high yields of biomass (8.88g/l) was achieved after seven days of incubation. The statistical analysis program One-way ANOVA test at significant level ($\alpha = 0.05$) showed that, there are highly significant differences ($p < 0.001$) within treatments of biomass dry weight and itaconic acid production. List significance differences LSD test revealed significant differences in the biomass dry weight means of *A. terreus* at all incubation periods and also significant differences are observed between itaconic acid production means at different incubation periods except (6 and 7) treatments. The obtained results were agreed with the results of Milson and Meers [3] and Roehr and kubicek [14] when they grew the fungus *A. terreus* on the molasses as a sugar source. They explained that high amount of itaconic acid production by the fungus *A. terreus* was obtained within 2 - 3 days of incubation.

3-Effect of Different Temperatures on Itaconic Acid Production by *A. terreus*

The amount of itaconic acid has been determined at different temperatures and the results are given in table (3). As shown, the highest amount and percent of itaconic acid production were determinate in culture medium at temperature of 33˚C which reached to 38.89g/l (756.11%). The lowest recorded of residual sugar (0.17%) were obtained with highest amount of accumulated itaconic acid in culture medium. and present variance in the final pH values at different temperatures. The results also indicated that the biomass dry weight was gradually increases by increasing the incubation temperature. And the high yielding was obtained at 36˚C. Statistical analysis revealed that, there are no significant differences ($p < 0.05$) within treatments in biomass dry weight and there are highly significant differences ($p < 0.001$) within treatments in itaconic acid production. And LSD test revealed that, there are significant differences in the biomass dry weight between different treatments except (30 and 33)˚C , (30 and 36)˚C and (33 and 36)˚C. While in case itaconic acid production there are significant differences between different treatments except (27and 36)˚C , (30 and 33)˚C. Similar results are previously described by Milson and Meers [15], Roehr and Kubicek [14], in which they explained that, the accumulation of itaconic acid by *A. terreus* in submerged culture fermentation containing molasses as carbon source occurred at 32 - 40˚C.

4-Effect of Nitrogen Level on Itaconic Acid Production by *A. terreus*

The nitrogen content of the medium was altered by varying the concentration of urea added to the medium. The organism was incubated for three days and the results presented in table 4.

The best amount and percent of itaconic acid production were detected in culture medium containing 0.3% urea which reached to 35.77g/l (541.68%).while the best concentration of the urea for the high yields of fungal biomass dry weight (7.42g/l) was 0.6%. Present variance in the final pH values at different concentrations of urea, and present variance in the residual sugar since the lowest recording of it were at concentration (0.7%) of urea. Statistical analysis revealed that, there are no differences within treatments in biomass dry weight, while there are highly significant differences ($p < 0.001$) within treatments in itaconic acid production. LSD

Days of Incubation	Dry Weight g/l	Itaconic Acid g/l	Itaconic Acid %	Residual Sugar %	Final pH
2	7.45 (0.01)	31.98 (0.00)	429.46	0.57 (0.06)	3.56 (0.006)
3	7.92 (0.07)	35.75 (0.00)	451.41	0.17 (0.06)	3.51 (0.006)
4	8.35 (0.05)	16.27 (1.09)	194.41	0.67 (0.06)	3.52 (0.006)
5	8.55 (0.04)	14.39 (1.09)	168.35	0.43 (0.06)	3.51 (0.03)
6	8.71 (0.05)	5.59 (0.00)	64.16	0.50 (0.1)	3.52 (0.006)
7	8.88 (0.04)	5.59 (0.00)	62.95	0.13 (0.06)	3.47 (0.006)

Table 2: Effect of incubation periods on itaconic acid production by *A. terreus.*

Each number represented the mean of 3 replicates and the number between brackets represented standard deviation (S.D).

test revealed significant differences in the itaconic acid production means at different urea levels except (0.5 and 0.6) %. This finding approximately agreed with the results obtained by Al-Mataiuty [8]. Who found that the highest production of itaconic acid by the fungus *A. terreus* was obtained at the concentration 0.35% of urea [16].

Temperatures ˚C	Dry Weight g/l	Itaconic Acid g/l	Itaconic Acid %	Residual Sugar %	Final pH
27	3.32 (0.48)	18.16 (4.35)	551.35	0.30 (0.1)	.370 (0.05)
30	5.25 (0.53)	33.24 (2.18)	635.74	0.23 (0.06)	3.60 (0.01)
33	5.16 (0.31)	38.89 (1.09)	756.11	0.17 (0.06)	3.52 (0.01)
36	5.53 (1.08)	15.64 (4.35)	289.25	0.23 (0.15)	3.45 (0.02)

Table 3: Effect of different temperatures on itaconic acid production by *A. terreus.*

Each number represented the mean of 3 replicates and the number between brackets represented standard deviation (S.D).

Urea %	Dry Weight g/l	Itaconic Acid g/l	Itaconic Acid %	Residual Sugar %	Final pH
0.00	6.47 (0.44)	17.55 (0.00)	272.00	0.77 (0.06)	3.76 (0.01)
0.20	7.31 (0.68)	21.32 (0.00)	293.21	0.50 (0.10)	3.62 (0.01)
0.30	6.61 (0.35)	35.77 (1.09)	541.68	0.60 (0.10)	3.53 (0.01)
0.40	7.07 (0.22)	26.35 (4.35)	378.82	0.57 (0.21)	3.53 (0.01)
0.50	6.88 (0.85)	13.78 (0.00)	202.35	0.63 (0.06)	3.57 (0.006)
0.60	7.42 (1.28)	13.78 (0.00)	189.51	0.53 (0.15)	3.60 (0.01)
0.70	7.36 (1.81)	10.01 (0.00)	141.82	0.20 (0.10)	3.68 (0.006)

Table 4: Effect of different concentration of urea on itaconic acid production by *A. terreus*

Each number represented the mean of 3 replicates and the number between brackets represented standard deviation (S.D).

Conclusion

The main aim of this study has come to true and been achieved, which is the benefit of carob pod extract as a raw material and carbon source for higher production of itaconic acid by the fungus *A. terreus*. The results of the present work also indicate that itaconic acid production by the fungus *A. terreus* is affected by incubation period, incubation temperature and concentration of added nitrogen source to the fermentation media.

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Volume 1 Issue 7 July 2018