

Bioproduction of itaconic acid exposed to some coumarins

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Abstract : Itaconic acid (2-methylidenebutanedioic acid) is an unsaturated di-carbonic acid. It has a broad application spectrum in the industrial production of resins and is used as a building block for acrylic plastics, acrylate latexes, super-absorbents, and anti-scaling agents. The efficacy of 6,8-difluoro-7-hydroxy-4-methylcoumarin and 6,8-difluoro-4-methyl umbelliferyl phosphate was studied on itaconic acid production by *Aspergillus terreus* SS-201. It has been found that both the coumarins under trial has stimulatory effect and has enhanced the yield of itaconic acid to an extent of 6.021% and 9.905% respectively in comparison to control.

(Keywords: Itaconic acid, coumarins and *Aspergillus terreus* SS-201.)

Introduction

Itaconic acid is an organic acid that is used as a platform chemical for the production of various value added chemicals such as poly-itaconic acid, resins biofuel components, ionomer cements etc. Itaconic acid and its derivatives have wide applications in the textile, chemical and pharmaceutical industries. The depletion of fossil fuels and the need for sustainable development require that fermentative itaconic acid production replace petroleumbased methods of itaconic acid production. Various microorganisms have been employed in itaconic acid fermentations, with the most prolific producer being *Aspergillus terreus*. Over 80 g/L itaconic acid has been produced in fermentations using glucose. However, there is an increasing interest in the utilization of lignocellulosic materials for itaconic acid production due to the concern of food security. Recent trends in itaconic acid research summarized in this paper indicate that itaconic acid can be

produced cost effectively from sustainable raw materials and have the potential to replace petro-based chemicals in various applications. Itaconic acid, or methylenesuccinic acid, is an organic compound. Itaconic acid is a white crystalline powder. Itaconic acid is a naturally occurring compound, non-toxic, and readily biodegradable. The name itaconic was devised as an anagram of aconitic. Historically, itaconic acid was obtained by the distillation of citric acid. Since the 1960s, it is produced industrially by the fermentation of carbohydrates such as glucose using *Aspergillus terreus*. As such, it is a fully sustainable industrial building block. It is primarily used as a co-monomer in the production of acrylonitrile-butadiene-styrene and acrylate latexes with applications in the paper and architectural coating industry.

The variety of physiological effects of natural coumarins is extensive. More than 500 coumarins exist in nature, although only a few are usefully found in any particular plant family¹⁻¹¹. Numerous physiological effect of coumarins are known but clear functions of these compounds generally remains to be unknown¹²⁻¹⁹. Literature survey reveals that a very few work has been done on efficacy of coumarins on fermentation²⁰ especially itaconic acid fermentation²¹⁻²³. Therefore, the authoress has employed some coumarins on itaconic acid production by *Aspergillus terreus* - SS-201

Experimental

The influence of 6,8-difluoro-7-hydroxy-4-methylcoumarin on itaconic acid production by *Aspergillus terreus* SS-201

The composition of the production medium for itaconic acid production by *Aspergillus terreus* SS-201 has been prepared as follows :

Glucose : 22.0 g ; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.35 g ;
 NH_4NO_3 : 0.90 g ; KCl : 0.05 g ; NaCl : 0.05 g ;
 KH_2PO_4 : 0.25 g ; pH : 2.2.

The pH of the production medium was adjusted to 2.2 by adding requisite amount of NH_4OH buffer solution. The production medium was sterilized in an autoclave maintained at 15 lbs steam pressure for about 15 minutes and cooled to room temperature.

The above composition medium represents volume of a fermentor flask, i.e., "100ml" production medium for itaconic acid production by *Aspergillus terreus* SS-201. Now, the same production medium for itaconic acid production by *Aspergillus terreus* SS-201 was prepared for 99-fermentor flask, i.e; each contained '100ml' of production medium.

The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged in 3-subsets, each consisting of 3-fermentor flasks. The remaining 9-fermentor flasks out of 99-fermentor flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermentor flasks.

After preparing the above sets of fermentor flasks M/100 solution of 6,8-difluoro-7-hydroxy-4-methylcoumarin was prepared and from the above coumarin solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above 1st to 10th sets respectively. The control fermentor flasks contained no coumarin.

Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of 6,8-difluoro-7-hydroxy-4-

methylcoumarin in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x} \text{ M}$ i.e., $1.0 \times 10^{-4} \text{ M}$: $2.0 \times 10^{-4} \text{ M}$, $3.0 \times 10^{-4} \text{ M}$, $4.0 \times 10^{-4} \text{ M}$, $5.0 \times 10^{-4} \text{ M}$, $6.0 \times 10^{-4} \text{ M}$, $7.0 \times 10^{-4} \text{ M}$, $8.0 \times 10^{-4} \text{ M}$, $9.0 \times 10^{-4} \text{ M}$ and $10.0 \times 10^{-4} \text{ M}$.

A = amount of chemical coumarin, i.e., 1.0 ml to 10 ml, x = Molarity of the coumarin soln.

The above fermentor flasks were then sterilized, cooled inoculated, incubated and analysed after 7, 8 and 9 days for itaconic acid formed²⁴ by *A. terreus* SS-201

The experimental procedure for the study of the influence of 6,8-difluoro-4-methylumbelliferyl phosphate to be incorporated in the production medium was exactly the same as described above with the only difference that in place of M/100 solution of 6,8-difluoro-7-hydroxy-4-methylcoumarin under trials 6,8-difluoro-4-methylumbelliferyl phosphate was incorporated into the production medium for itaconic acid production by *Aspergillus terreus* SS-201

Results and Discussion

The data recorded in the table-1 shows the influence of 6,8-difluoro-7-hydroxy-4-methylcoumarin on itaconic acid production by *Aspergillus terreus* SS-201. The results show that the compound 6,8-difluoro-7-hydroxy-4-methylcoumarin is not much beneficial and encouraging for the itaconic acid production by *Aspergillus terreus* SS-201 and thus causes slight enhancement in the production of itaconic acid exposed to coumarin concentrations from 1.0×10^{-4} to $10.0 \times 10^{-4} \text{ M}$.

The maximum yield of Itaconic acid was found to be at $7.0 \times 10^{-4} \text{ M}$ concentration of 6,8-difluoro-7-hydroxy-4-methylcoumarin, i.e., 4.243g/ 100 ml in 8 days of optimum incubation period which is 6.023% higher in comparison to control fermentor flasks, i.e., 4.002g/ 100 ml in the same

Table - 1
Study on itaconic acid production by *Aspergillus terreus*
exposed to 6,8-difluoro-7-hydroxy-4-methylcoumarin

Concentration of coumarin used	*Yield of itaconic acid in g/100ml			% Difference in the yield of Itaconic acid after 8days
	7 days	8 days	9 days	
Control – coumarin	2.135	4.002	3.540	–
1.0 x 10 ⁻⁴ M(+) coumarin	2.163	4.053	3.578	(+) 1.274
2.0 x 10 ⁻⁴ M(+) coumarin	2.180	4.062	3.583	(+) 1.499
3.0 x 10 ⁻⁴ M(+) coumarin	2.189	4.078	3.590	(+) 1.899
4.0 x 10 ⁻⁴ M(+) coumarin	2.195	4.094	3.600	(+) 2.298
5.0 x 10 ⁻⁴ M(+) coumarin	2.215	4.120	3.639	(+) 2.948
6.0 x 10 ⁻⁴ M(+) coumarin	2.230	4.215	3.675	(+) 5.322
7.0 x 10 ⁻⁴ M(+) coumarin**	2.235	4.243**	3.695	(+) 6.021
8.0 x 10 ⁻⁴ M(+) coumarin	2.229	4.129	3.631	(+) 3.173
9.0 x 10 ⁻⁴ M(+) coumarin	2.218	4.109	3.603	(+) 2.673
10.0 x 10 ⁻⁴ M(+) coumarin	2.197	4.095	3.599	(+) 2.323

Table - 2
Study on itaconic acid production by *Aspergillus terreus*
exposed to 6,8-difluoro-4-methylumbelliferyl phosphate

Concentration of coumarin used	*Yield of itaconic acid in g/100ml			% Difference in the yield of Itaconic acid after 8days
	7 days	8 days	9 days	
Control – coumarin	2.139	4.008	3.549	–
1.0 x 10 ⁻⁴ M(+) coumarin	2.236	4.110	3.641	(+) 2.544
2.0 x 10 ⁻⁴ M(+) coumarin	2.321	4.195	3.726	(+) 4.665
3.0 x 10 ⁻⁴ M(+) coumarin	2.417	4.295	3.810	(+) 7.160
4.0 x 10 ⁻⁴ M(+) coumarin	2.467	4.345	3.860	(+) 8.408
5.0 x 10 ⁻⁴ M(+) coumarin**	2.527	4.405**	3.920	(+) 9.905
6.0 x 10 ⁻⁴ M(+) coumarin	2.329	4.362	3.862	(+) 8.832
7.0 x 10 ⁻⁴ M(+) coumarin	2.280	4.275	3.785	(+) 6.661
8.0 x 10 ⁻⁴ M(+) coumarin	2.259	4.233	3.748	(+) 5.613
9.0 x 10 ⁻⁴ M(+) coumarin	2.211	4.143	3.668	(+) 3.368
10.0 x 10 ⁻⁴ M(+) coumarin	2.181	4.086	3.618	(+) 1.946

* Mean of three observations, ** Optimum concentration of coumarin

*** Optimum yield of itaconic acid, (+)ve values indicate % increase in the yield of itaconic acid
 Experimental deviation ($\pm$) 1.5% to 3.5%

set of experimental parameters.

The results of the influence of 6,8-difluoro-4-methylumbelliferyl phosphate on itaconic acid production by *Aspergillus terreus* SS-201 has been recorded in the table-2. It is obvious from the results that the presence of above coumarin in the production medium has also enhanced the yield of itaconic acid at its all molar concentrations.

It has been noticed that the presence of above coumarin compound, i.e., 6,8-difluoro-4-methylumbelliferyl phosphate was found to be beneficial and encouraging for the improved yield of itaconic acid up to its concentration from

1.0×10^{-4} M to 5.10^{-4} M but not so significant and above this concentration the coumarin under trial has retarded the yield of Itaconic acid. However, it is interesting to note that itaconic acid production by *Aspergillus terreus* SS-201 exposed to all the experimental coumarin concentration, i.e., from 1.0×10^{-4} M to 10.0×10^{-4} M of coumarin under trial has been found to be slight better than the control fermentor flask.

The maximum itaconic acid production was observed at 5.0×10^{-4} M concentration of 6,8-difluoro-4-methylumbelliferyl phosphate, i.e., 4.405g/100ml which is 9.905% higher in comparison to control fermentor flasks, i.e., 4.008 g/100 ml in 8 days of optimum incubation period.

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