

Studies on itaconic acid production by submerged fermentation process exposed to some micelles

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Abstract : Itaconic acid is an unsaturated organic acid with two carboxyls and one methyl group. The presence of these functional groups, along with a conjugated double bond makes itaconic acid a versatile molecule with a vast number of applications. In the present communication the authoress has studied efficacy of some micelles i.e. potassium octyl sulfate on biological production of itaconic acid by *Aspergillus terreus* SS-201. It has been found that the micelles under trial i.e. potassium octyl sulfate has beneficial impact and has enhanced yield of itaconic acid to an extent of 16.685% higher in comparison to control.

(Keywords : Itaconic acid fermentation process, potassium octyl sulfate and *Aspergillus terreus* SS-201)

Introduction

Micelles are self-assembling amphiphilic molecule consisting of hydrophobic cores and a hydrophilic capsule and can potentially carry hydrophobic drugs encapsulated in the core and enhance their bioavailability. Micelles have been studied as drug delivery carriers for decades.

Many different drug carriers have been developed for controlled drug delivery in recent decades, including micelles, liposomes, polymer or protein-drug conjugates, polymeric nanoparticles, and pathogens¹⁻¹³. Among these drug carriers, micelles have a number of attractive features. Micelles are self-assembled microstructures formed by surfactants in an aqueous system and are usually < 50 nm in diameter¹⁴.

A tiny particle made of substances that are soluble in water and that come together to form a ball like shape. These particles can carry other substances inside them. Today, science has found various practical applications for micelles. The properties of surfactant substances in aqueous media, been studied by scientists and there are currently several practical applications of the phenomenon of micelles formation.

The process of forming micelles is known as micellisation and forms part of the phase behaviour of many lipids according to their polymorphism¹⁵⁻¹⁷. A number of recent review articles have been written on the rheology and applications of wormlike micelle solutions¹⁸⁻³⁰.

Thus, from the above brief review it is evident that micelles are required for exploitation specially for Itaconic acid fermentation and in view of this the authoresses has studied the influence of potassium octyl sulfate on itaconic acid production by *Aspergillus terreus* SS-201

Experimental

The influence of potassium octyl sulfate on itaconic acid production by novel *Aspergillus terreus* SS-201.

The composition of the production medium for itaconic acid production by novel *Aspergillus terreus* SS-201 isolate 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 : 0.05g ;
 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" for itaconic acid production by novel *Aspergillus terreus* SS-201 isolate.

Now, the same production medium for itaconic acid production by novel *Aspergillus terreus* SS-201 isolate 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/1000 solution of potassium octyl sulfate was prepared and from the above micelle 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 micelles.

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 potassium octyl sulfate 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., $1.0 \times 10^{-5} \text{ M}$ to $10.0 \times 10^{-5} \text{ M}$

Where

A = amount of chemical micelle in ml

x=Molarity of the micelle solution respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 32°C

and analysed after 7, 8 and 9 days for itaconic acid³¹ formed.

Results and Discussion

The data recorded in the table 1 shows that potassium octyl sulfate has stimulatory effect on the itaconic acid production by novel *Aspergillus terreus* SS-201 isolate.

The maximum yield of itaconic acid, i. e. 5.140g/100 ml in the presence of potassium octyl sulfate was observed at $7.0 \times 10^{-5} \text{ M}$ molar concentration in 8 days of optimum incubation period which is 16.685% higher in comparison to control fermentor flasks, i.e; 4.405 g/100 ml in the same times course and other same experimental parameters.

The higher molar concentrations of potassium octyl sulfate were not much favourable for the itaconic acid production by novel *Aspergillus terreus* SS-201 isolate. So the gradual addition of the micelle potassium octyl sulfate after certain concentrations were not beneficial for the itaconic acid fermentation process.

It has been observed that molar concentration of the micelle i.e., potassium octyl sulfate from $1.0 \times 10^{-5} \text{ M}$ to $8.0 \times 10^{-5} \text{ M}$ enhances the yield of itaconic acid to a certain order being 0.567%, 0.953%, 3.246%, 4.015%, 10.356%, 10.624%, 16.685% and 12.009% higher in comparison to control flasks but at $9.0 \times 10^{-5} \text{ M}$ to $10.0 \times 10^{-5} \text{ M}$ the yield of itaconic acid shifted to be in lower range, i.e., 7.514% and 2.383% higher in comparison to previous concentrations of potassium octyl sulfate taken into experimental trials.

It has been observed further that after optimum concentration, i. e., $7.0 \times 10^{-5} \text{ M}$, the addition of the same micelle to the production medium causes fall in the yield of itaconic acid gradually and reaches to 2.383%; However, at all the experimental concentrations of potassium

Table - 1
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Concentration of micelle used	*Yield of itaconic acidin g/100mL			% Difference in the yield of Itaconic acid after 8 days
	7 days	8 days	9 days	
Control – micelle	2.540	4.405	3.710	–
1.0 x 10 ⁻⁵ M + micelle	2.552	4.430	3.728	(+) 0.567
2.0 x 10 ⁻⁵ M + micelle	2.562	4.472	3.743	(+) 9.53
3.0 x 10 ⁻⁵ M + micelle	2.616	4.548	3.821	(+) 3.246
4.0 x 10 ⁻⁵ M + micelle	2.641	4.582	3.858	(+) 4.018
5.0 x 10 ⁻⁵ M + micelle	2.692	4.685	3.932	(+) 6.356
6.0 x 10 ⁻⁵ M + micelle	2.794	4.873	4.081	(+) 10.624
7.0 x 10 ⁻⁵ M + micelle **	2.946	5.140***	4.303	(+) 16.685
8.0 x 10 ⁻⁵ M + micelle	2.844	4.934	4.155	(+) 12.009
9.0 x 10 ⁻⁵ M + micelle	2.717	4.736	3.969	(+) 7.514
10.0 x 10 ⁻⁵ M + micelle	2.590	4.510	3.784	(+) 2.383

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

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

octyl sulfate used for the itaconic acid production by novel *Aspergillus terreus* SS-201 isolate has been found higher in comparison to control fermentor flasks.

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