STIMULATION OF CEPHALOSPORIN PRODUCTION DURING THE FERMENTATION OF CEPHALOSPORIUM ACREMONIUM IMMOBILIZED IN PVA-ALGINATE BEADS BY ULTRASONICATION

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ABSTRACT

Production of antibiotics is one of the most important areas in the field of applied microbiology and cephalosporin is one of the best-used antibiotics till today. Conventional methods of fermentation for more productivity are by immobilization and bioreactor studies. During recent years a drive towards "process intensification" is leading to the search for diverse fields like sound, microwave, photoenergy etc to provide unusual environment for the enhanced product formation. In this view, a study has been undertaken to see the effect of sonication on the cephalosporin formation by C. acremonium ATCC 48272 immobilized in PVA-Alginate beads compared with that of control. Sonication at 10 KHz for 10 min; 28^{0}C showed that sonication of fermented medium at every 24 hrs is effective, which gave a yield of 418.23 μg/ml and almost remained stable for 8 days without much decline in yield of the product; when compared to control & if sonication was stopped at 24 hrs, drop in the yield was noticed. Sonication during growth stage plays a negative role because early stages of growth cells are sensitive to sonication, but at production stage higher yield was noticed due to cavitation effect. Ultrasonication improved both external & internal mass transfer. Enhancement in cephalosporin production is discussed in detail.

Keyword: *Cephalosporin, immobilization, ultrasonication, C. acremonium.*

1. INTRODUCTION

Ultrasound used in a liquid medium for spheroblastlysis & for dispersing the fine particulate matter in centrifuge pellets, and for cell disruption. In microbiology ultrasonication is mainly used for cell disruption. Ultrasonication causes cavitation, the formation of minute bubbles of gas or vapour in those regions of the liquid corresponding to rare fractions in the sound waves. Ultrasound may induce phonophoretic effects across cell walls and membranes of microbes-[1].

Ultrasound when applied at a optimum level and proper dosage to a suspension of free / immobilized microbial cells which preserves their structural integrity under these conditions. Phonopheresis [2] is a process whereby ultrasound facilities the penetration of chemicals through membranes in artificial and biological systems. This process may enhance diffusion of foreign molecules, resulting in an enhanced rate of the bioprocess. Studies related to ultrasound can be used as an acceleration of diffusion process, where the activity of immobilized cells are

stimulated by ultrasound was investigated by [3]. The antimicrobial activity of many antibiotics penicillin, streptomycin, ampicillin etc and bactericides was enhanced by 1.7-10.8 fold by exposure of the culture to low frequency ultrasound [4], [5] have investigated the effect of ultrasound on mass transfer during cheese bringing. Many studies which improved the development of sonochemistry including the function mechanisms, application and its research status has been well understood. This study investigated the effects of ultrasonic treatment on cephalosporin production during fermentation process using PVA-Alginate beads as the biocatalyst.

2 MATERIALS & METHODS

2.1. Organism and Media

C. acremonium ATCC 48272 is used throughout this study. Spore stocks are maintained on a sporulation medium containing (in g/L); Soluble starch, 15; Yeast extract, 4.0; K_2HPO_4, 1.0; $MgSO_4$,1.0; pH:6.5. The growth phase medium contained (in g/L); Peptone, 20; Malt extract, 20; Corn steep liquor, 5.0; $MgSO_4$, 0.25; K_2HPO_4, 0.5; KH_2PO_4, 1.0; $CaCl_2$, 0.1. pH maintained at 6.5± 0.2 with NaOH / HCl. The defined production phase medium for bioreactor contained (in g/L); Sucrose, 80; Soya-bean meal, 60; $CaCO_3$, 1.5; DL-Methionine, 7.0; ammonia, 30. Sucrose was autoclaved separately; pH 6.0.

2.2 Immobilization procedure

Dissolve 16% poly vinyl alcohol in distilled water and to this add 2% of alginate. To the co.polymer solution of PVA-alginate; 3% of spore suspension containing $8.4x10^8$ spores /ml were suspended in sterile condition and the suspension was added drop wise to 0.1 $CaCl_2$. The PVA-alginate beads were kept for overnight in this solution at 4^0C then washed with saline and further subsequent washes by using distilled water. All materials used were sterilized in an autoclave. 10% beads are used for inoculation & incubated in growth medium for 5 days at 27ºC, and transferred to production medium.

2.3 Ultrasonic treatment

Ultrasound is generated using a bath type sonicator "Decon" at a frequency of 10 KHz with a controlled treatment time for 10 min at 28^0C, which is tuned automatically.
Expt-1: beads are sonicated before growth is initiated.
Expt-2: beads are sonicated during later stages of growth
Expt-3: beads are sonicated before production was initiated only at "24" hours.
Expt-4: beads are sonicated during production stage at every 24 hrs starting from "0"hrs onwards.
Expt-5: control - without any sonication Samples were collected for cephalosporinestimation at every 24 hrs by microbiologicalassay [6].

3 RESULTS AND DISCUSSION

Impact of sonication on cephalosporin production is conducted at different levels and compared with that of control (unsonicated).It is seen from the table -1 that product yield is affected when the sonication is carried out at growth stages, i.e. experiments 1 & 2. Thus sonication during growth stage has negative role because during the early or later stages of growth the cells are sensitive to sonication and not capable to grow actively. Hence, cells are weakly grown due to shear & not capable for producing good yield of cephalosporin. Exp.: 4 is carried to allow more flexible control over sonication by subjecting a sonication pulse for 10 min (10 KHz) at every 24 hours interval, which showed more active fermentation with higher yields by maintaining the viability of cells when compared to exp. 3. In exp. 3, cells were radiated with sonic waves only at 24hrs and stopped from further dosesof sonication, which caused no stimulation in fermentation process and appeared to decline in cephalosporin production thereafter.

Table: 1 Effect of ultrasonic treatment on cephalosporin production from *C.acremonium.*

Time (hrs)	Cephalosporin production (µg/ml) at every 24 hrs of incubation.				
	Exp1	**Exp.2**	**Exp.3**	**Exp.4**	**Exp5**
0	0	0	0	0	0
24	148.7	119.5	236.5	251.2	148.7
48	163.4	67.80	192.6	265.8	178.0
72	192.6	107.0	221.9	418.2	192.6
96	148.7	67.80	178.0	295.1	205.1
120	134.1	67.80	119.5	295.1	178.0
144	134.1	67.80	119.5	236.5	148.7
168	119.5	67.80	134.1	251.2	134.1
192	109.1	67.80	119.5	250.4	131.2

Sonication carried out during production stage has a positive role in increasing production of secondary metabolite, this is because the chain cells are broken into single individual cells and easily takes up the nutrients and releases the products with much ease. Figure 1 indicates the comparison drawn between control (expt#5) with that of (expt#4) i.e. treated with ultrasonication for 10 min/24hrs cycle is almost effective for enhancing the level of cephalosporin and maintaining the stability in yield. Continuous exposure at 10-min/24 hrs cycle did not destroy cephalosporin activity and this treatment would be expected to progressively increase the hydrated surface area available for fermentation. The basis for this detrimental effect appears to reside in both the fermentation process and the sensitivity of biocatalyst. Continuous exposure to ultrasound was not lethal for *C. acremonium* and effectively enhanced sugar metabolism, growth and cells division. This may be due to increased metabolite biosynthesis. Thus the ultrasound treatment for a period of 24 hours cycle may be required by the biocatalyst for active cell divisions resulting in improved fermentation process.As seen , from figure: 1 distinct enhancement was observed with sonicated cells over the unsonicated immobilized cells but it is not the same for free cells. Cells embedded within the gel beads provided better protection to the biocatalysts in an ultrasonic field and consequently harsher sonication regimes could be applied, unlike the milder ones employed in free cells systems. Yet the sonication applied is such that no damage occurred to the gel beads.

In our system, the observed ultrasound induced effect is significant in the gel beads this may be due to the diffusivity of the molecules is quite slower in a solid medium such as the gel beads, a further facilitation of the substrate diffusion could be more easily achieved especially in the outer shell of the beads. This could explain why ultrasound induced effect is more prominent in the immobilized cell system. Oxygen limitation, especially in immobilized cell systems is a common phenomenon in oxygen consuming processes and ultrasound has most likely facilitate its diffusion into the gel beads. Free cells when treated with ultrasonication caused dispersal of microbial clumps but simultaneously inhibited the cephalosporin production; this may be due to damage caused to the cells.

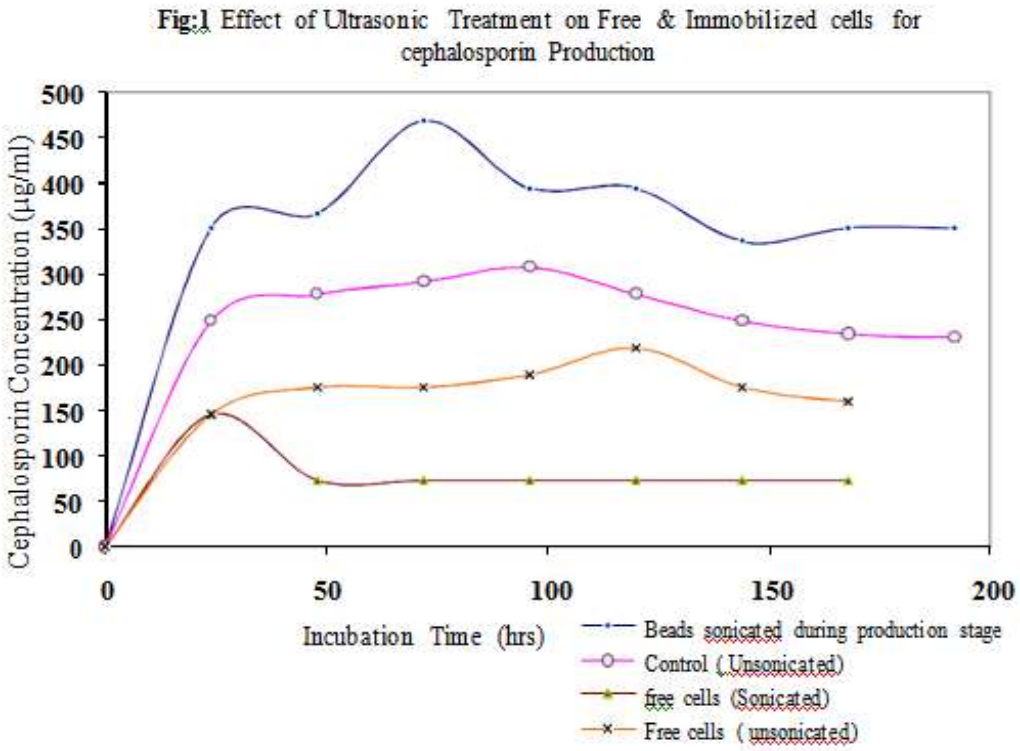

Fig. 1 Effect of Ultrasonic Treatment on Free And Immobilized Cells For Cephalosporin Production

Ultrasonic cavitation is a complex and dynamic phenomenon which is influenced by many factors including temperature, dissolved gases, suspended particulate, proximity of resonator to the nucleating surfaces etc [7]; [8]. Thus it is likely that high intensity mixing at the particle surface caused by ultrasound also increased the dissociation of substrates and allow active cells to rebind at new sites which are productive for continued fermentation [9]. Sonicwaves may have two causes one is itsmechanical action to make the solid substrates fine by inter attrition of these suspended particles, the other is cavitation effect to facilitate the fine substrate through the cellular membrane. Ultrasound improves both external and internal mass transfer [10]. In this study exp.: 4 performed well in which the higher cephalosporin yield of 418.23 µg/ml at 72 hours is obtained when compared to 205.1 µg/ml at 96 hours in the control experiment.

4 CONCLUSION

Sonication enabled the catalytic activity of the microbial cells and also retained their viability. It can be concluded that the sonication regime was not harsh enough and had induced effects for immobilized beads. Analogous effects on both mycelia and dissociation of nutrient components may also occur in the intensive mass transfer, which achieved extremely high rates ofcephalosporin production.

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