

## Stimulation of Cephalosporin Production during the Fermentation of *CEPHALOSPORIUM ACREMONIUM* immobilized in Agar Beads by Ultrasonication

A. Swaroopa Rani

Centre for Biotechnology, Jawaharlal Nehru Technological University Hyderabad,

Kukatpally, Hyderabad – 500 085, Telangana (India).

E-mail: bioswar@yahoo.com

[Received-06/12/2014, Accepted- 18/12/2014, Published-21/03/2015]

### 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 20339 immobilized in agar beads compared with that of control.

Sonication at 10 KHz for 10 min; 28<sup>o</sup>C showed that sonication of fermented medium at every 24 hrs is effective, which gave a yield of 466.51 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.

**Key words:** cephalosporin, immobilization, ultrasonication, *C. acremonium*.

### [I] INTRODUCTION

Ultrasound used in a liquid medium for spheroblast lysis & 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 rarefractions in the sound waves. Ultrasound may induce phonophoretic effects across cell walls and membranes of microbes [2].

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 [10] is a process whereby ultrasound facilitates 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 [7].

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 [9], [8] 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 agar beads as the biocatalyst.

## [II] MATERIALS & METHODS

### 2.1. Organism and Media

*C. acremonium* ATCC 20339 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  $6.5 \pm 0.2$  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

Aqueous spore suspension  $8.4 \times 10^8$  spores/ml of 10.5ml is added to 2% agar solution swirled to obtain a homogeneous mixture; poured into paraffin oil in the bead form with the help of peristaltic pump.

The suspension is cooled to below the setting temperature of agar. After the beads had solidified, preparation is allowed to settle overnight at 30°C. Oil layer is decanted. Beads were filtered, washed with phosphate buffer solution and second time with water. All materials used were sterilized in an autoclave. 10% beads are used for inoculation & incubated in growth medium for 5 days at 27°C, 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°C, 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 cephalosporin estimation at every 24 hrs by microbiological assay [3].

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	248.7	219.5	336.5	351.2	248.7
48	263.4	87.80	292.6	365.8	278.0
72	292.6	117.0	321.9	466.5	292.6
96	248.7	87.80	278.0	395.1	307.3
120	234.1	87.80	219.5	395.1	278.0
144	234.1	87.80	219.5	336.5	248.7
168	219.5	87.80	234.1	351.2	234.1
192	209.1	87.80	219.5	350.4	231.2

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

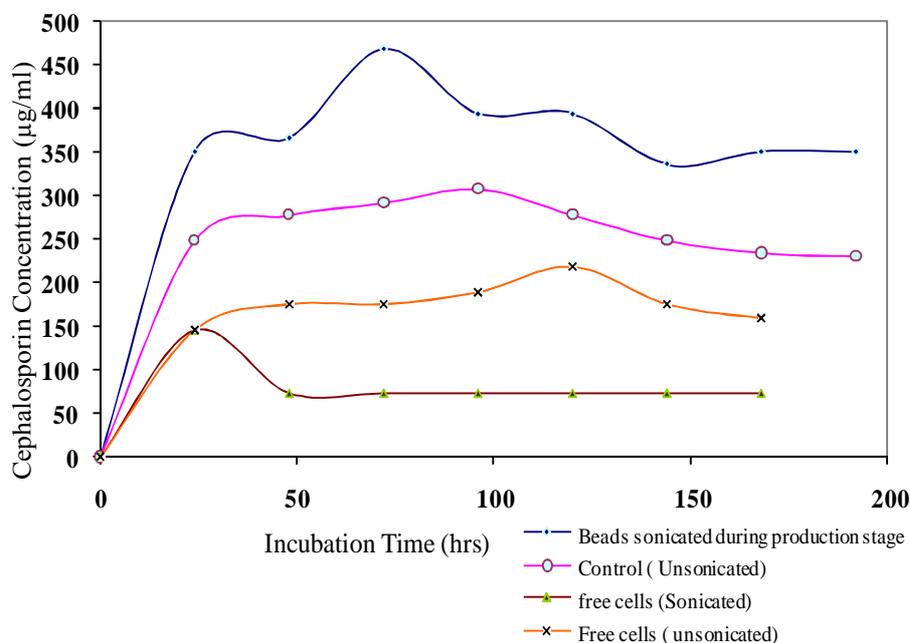
### [III] RESULTS AND DISCUSSION

Impact of sonication on cephalosporin production is conducted at different levels and

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 doses of sonication, which caused no stimulation in fermentation process and appeared to decline in cephalosporin production thereafter.

**Fig:1** Effect of Ultrasonic Treatment on Free & Immobilized cells for cephalosporin Production



compared with that of control (unsonicated). It is seen from the table -1 that product yield is

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.

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 [1]; [6]. 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 [4].

Sonicwaves may have two causes one is its mechanical 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 [5].

In this study exp.: 4 performed well in which the higher cephalosporin yield of 466.51 µg/ml at 72 hours is obtained when compared to 307.3 µg/ml at 96 hours in the control exp.

#### [IV] 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 of cephalosporin production.

## ACKNOWLEDGEMENT

The author thanks the University Grants Commission (UGC) for the financial support.

## REFERENCES

1. Atchely, L.A; Crum A. Acoustic cavitation and bubble dynamics. In ultrasound: its chemical, physical and biological effects; suslick, K.S. Ed; VCH. New York ; PP1-20. 1988.
2. Bar, R., Biotechnol, Bioengg. 32:655 1988.
3. Bandhopadhyay B, Humphrey AE, Taywkhii, M. Biotechnol, Bioeng. 9 : 533-544 1967.
4. Gusakov, A.V. Sinitsyn, A.P: Davydkim, I.Y; Davydkin, V.Y; protas,o.u. Enhancement of enzymatic cellulose hydrodynamics using a novel type of bioreactor with intensive stirring induced by electromagnetic field. Appl. Biochem Biotechnol., 56,141-153. 1996.
5. Mason, Timothy.J; Editor (JAI; Stanford,comm),.311pp. 1999.
6. Price, G.J. Introduction to sonochemistry. In current trends in sonochemistry, Price G.J. Ed, Royal society of chemistry special publication No. 116; Royal society of chemistry, Cambridge, U.K.; PP1-7 1992.
7. Schellenberfer, Alfred; Schmidt, Peter; Rosenfeld, Eike, Millner, Rudolf, Manusfeld, Johanna. Ger (East), 6 Feb, Appl. 253, 352; 1985.
8. Scanche, Emma S; Simal, Susana; Antony, Ros Sello, Forsch. A, 209 (3-4), 215-219. 1999.
9. Ukhov, A.Ya; Petrus, V.S.; Shvaidetskaya, G.V; Yatskevich, Ya.E. Antibiot, Med., Biotechnol., 30(9), 684-7. 1985.
10. Williams, A.R.. Ultrasound, Biological effects and potential hazards, Academic, London, PP. 177-253. 1983.