

## Enhanced Antimicrobials Production by *Streptomyces sundarbansensis* sp. nov. in a Novel Extended Surface Biofilm Reactor

Sreyashi Sarkar

Department of Chemical Engineering,  
Jadavpur University, Kolkata 700 032, India  
E-mail sreyoshisarkar3@gmail.com, Tel. 0091 33 2418 2866

[Received 04/12/2014, Accepted-22/12/2014]

### ABSTRACT

A newly designed extended surface biofilm reactor (ESBR) with an inner configuration consisting of eight equidistantly spaced polymethylmethacrylate rectangular strips placed radially on a circular disk to provide additional surface area for microbial growth was used for antimicrobials production by the biofilm-forming marine isolate *Streptomyces sundarbansensis* sp nov.. Antimicrobials production by *Streptomyces sundarbansensis* sp nov. is studied in small scale polymethylmethacrylate conico-cylindrical flask (PMMA-CCF), as well as in a 4.2 L ESBR. The best pH and temperature for antimicrobials production are determined (pH 10, 30°C) through a designed experimental study using PMMA-CCF. Consequently, operating conditions in the ESBR are studied using a 3<sup>rd</sup> experimental design wherein each of two (n = 2) parameters viz. aeration and inner configuration submergence are considered at three levels viz. high, medium, and low: 0.75, 0.50, and 0.25 L/min for aeration rate; and 75, 50, and 25 % for inner configuration submergence, (while maintaining the rotational speed at 140rpm). The 50% submergence condition together with maximum permissible aeration is found to be most favorable for antimicrobials production - peak antimicrobial activity (PAMA) attaining its highest value, 47 mm. PAMA increases with increasing aeration at all operating conditions studied - particularly, at 50% submergence condition, a threefold increase in aeration rate (0.25~0.75 L/min) causes PAMA to increase by 74%, thus showing the strong aeration dependence of this antimicrobials producer. Again, compare to the best values obtained in the 420 mL PMMA-CCF experiments, corresponding ESBR value is 15% higher for PAMA – strong evidence for applying this novel bioreactor for cultivation of antimicrobial-producing marine microbes.

**Keywords:** *Streptomyces*, antimicrobial, biofilm, polymethylmethacrylate

### 1. INTRODUCTION

The intertidal zone, which occupies the upper edge of the world's coasts extending over 1,600,000

km, is probably the most significant coastal habitat given its biological productivity and economic

value. It comprises rocky platforms, sandy beaches, mudflats, estuaries, salt marshes, mangrove forests, certain coral reefs and humanmade infrastructures. Intertidal microbial communities often occur as biofilms which are high-density attached communities embedded in extracellular biopolymer matrices [5]. The microbial biofilm is a common adaptation of natural bacteria and other microorganisms. In the fluctuating environments of intertidal systems, biofilms form protective microenvironments and may structure a variety of microbial processes [3]. Surface attachment and biofilm formation are known to influence metabolite production by microorganisms. Marine surface associated microorganisms may require conditions that resemble their native environment in order to produce the maximum amount of bioactive metabolites. For example, several studies have shown an increased production of antimicrobial compounds when the surface associated bacteria were grown, *in vitro*, to form surface attached biofilms [6].

Investigations by Yan et al. (2002) [15] showed that biofilm-forming bacteria produce important metabolites under surface-attached conditions. The modified roller bottle cultivation can be used to increase production of important metabolites [15]. Another new configuration, the air-membrane surface (AMS) bioreactor, designed by Yan et al. (2003) [16], allowed bacteria to grow as a surface-attached biofilm in contact with air. Results obtained showed that specific molecules are produced only when the producer microbes can grow as biofilms. Several studies were conducted in a rotating disc bioreactor that mimicked the intertidal habitat of three estuarine isolates, supported biofilm formation as well as production of antimicrobial metabolites, in particular, actinomycin D [14, 13, 10]. Based on these studies, a novel shaking flask, the polymethylmethacrylate conico-cylindrical flask (PMMA-CCF), with an inner arrangement that promotes biofilm formation and has a diameter

close to that of a 500-mL Erlenmeyer flask so that it can be easily placed in a rotary shaker for routine small-scale studies was designed [11, 12]. This small-scale vessel (420 mL) that provides significantly enhanced surface area as well as property for surface attachment of biofilm-forming microorganisms was successfully employed for the production of antimicrobials by intertidal actinobacteria *Streptomyces sundarbansensis* sp. nov. [1, 8, 2, 9].

There is, however, a lack of research into bioreactor engineering and fermentation protocol design in the field of marine bacterial antibiotic production. Most production strategies are carried out at the shake-flask level and lack a mechanistic understanding of the antibiotic production process, offering poor prospects for successful scale-up [4]. The area in which bioprocess engineering has had the greatest impact and still has the greatest opportunity for novel developments is in the design and optimization of bioreactors for marine metabolite production [7].

A novel bioreactor design, extended surface biofilm reactor (4.2 L) is proposed from the PMMA-CCF (420 mL) studies of *Streptomyces sundarbansensis* sp. nov., that also provide significantly increased surface area for the attachment of biofilm-forming microbes that ultimately enhanced metabolites production.

In this paper, the use of extended surface biofilm reactor (ESBR) for the production of antimicrobials by intertidal actinobacteria *Streptomyces sundarbansensis* sp. nov. is reported. The two process parameters that are considered for ESBR operation are submergence level of the inner configuration and aeration rate, that are varied simultaneously, viz. at three levels: high, medium, and low, to formulate  $3^n$  (with  $n = 2$ ) experimental design.

Now, the peak antimicrobial activity (PAMA) provides a direct measure of the maximum level of antimicrobials production for the above mentioned experimental design.

## 2. MATERIALS AND METHODS

### 2.1. Microorganisms and media composition

The marine isolate, a salt-tolerant estuarine actinobacterial strain *Streptomyces sundarbansensis* sp nov., designated as MS1/7<sup>T</sup>(=MTCC 5272<sup>T</sup>) [1] was sourced from the intertidal regions of the Sundarbans delta off the Bay of Bengal. The novel actinomycete produces a cytotoxic antimicrobial of molecular weight 300.2 and predicted molecular formula C<sub>20</sub>H<sub>28</sub>O<sub>2</sub> [8] and also 2-allyloxyphenol [1, 2] at shake flask level. The compound inhibits three Gram-positive and three Gram-negative multiple drug resistant (MDR) bacteria (defined as that disease-causing bacterium which can survive in the presence of structurally unrelated antimicrobial compounds targeted at distinct bacterial physiological mechanisms to eradicate the disease causing bacterium), seven non-clinical Gram-positive, four Gram-negative bacteria and five fungi [8].

The strain was maintained on an enrichment medium (EM) (all units in g/L, K<sub>2</sub>HPO<sub>4</sub> 0.5, casein 3.0, starch 10.0, peptone 1.0, yeast extract 1.0, malt extract 10.0 and agar 15.0, distilled water 500 ml and natural seawater 500 ml, pH 7.4) [10] slant, stored at 4 °C, and subcultured every month. Sucrose based medium (SBM) (all units in g/L, starch 2.0, soybean meal 2.0, yeast extract 0.5, CaCO<sub>3</sub> 0.32, CuSO<sub>4</sub> 0.005, MnCl<sub>2</sub> 0.005, ZnSO<sub>4</sub> 0.005, sucrose 170 g/L, seawater 500 ml, distilled water 500 mL, pH 10 was used as the production medium and 100.0 mL of 9 x10<sup>9</sup> CFU/ml spore and substrate mycelium suspension was used as inoculums for 2L of the said production medium.

### 2.2. Construction of the extended surface biofilm reactor (ESBR) and cultivation conditions

The ESBR, which helps microbial growth in the form of surface-attached biofilms, was designed based on the concept of the PMMA conico-cylindrical flask [11, 12] as shown in Fig. 1a and Fig. 1b. The maximum volume of the ESBR is 4.2 L. and the inner configuration (on which eight equispaced rectangular strips were placed on a

circular disk) was rotated by the use of magnetically coupled motor as shown in Fig. 2. The surface of the inner configuration was roughened with Grade 50 sand paper [13]. The entire ESBR was made of polymethylmethacrylate (PMMA) which is transparent, corrosion-resistant to high salt concentrations, and generally provides a surface conducive to attachment of biofilm. The ESBR was fabricated by Plastic Abhiyanta, Kolkata, India.

For sterilization, the ESBR was disassembled, the components washed thoroughly in tap water and immersed in 3% (v/v) sodium hypochlorite for 5 h. The parts were then surface-sterilized under UV light (TUV15 W/G15T8, Philips, The Netherlands) in a laminar airflow bench for 30 min [11].

The production medium was sterilized ex situ and added aseptically to the reactor disinfected with repeated washing with sodium hypochlorite and UV exposure.

Sterile air was supplied into the ESBR using an aquarium pump. The supplied air was made sterile using a sterile cotton plugged pipe that uniformly dispersed into the medium.

Ports on the top lid of the reactor were available for sampling, addition of medium/inoculum/antifoam, pH, dissolved oxygen, temperature sensors, air inlet and exhaust.

Submergence level of the inner configuration and aeration rate was varied simultaneously, viz. at three levels: high, medium, and low i.e. 75%, 50% and 25% for submergence level and 0.75 L/min, 0.50L/min and 0.25L/min for aeration level, to formulate 3<sup>n</sup> (with n = 2) experimental design. Samples were aseptically withdrawn at 2h interval and the ESBR was operated for 96h in a batch mode.

The ESBR was run twice, and the results were reproducible within less than 3% deviation.

### 2.3. Analytical methods

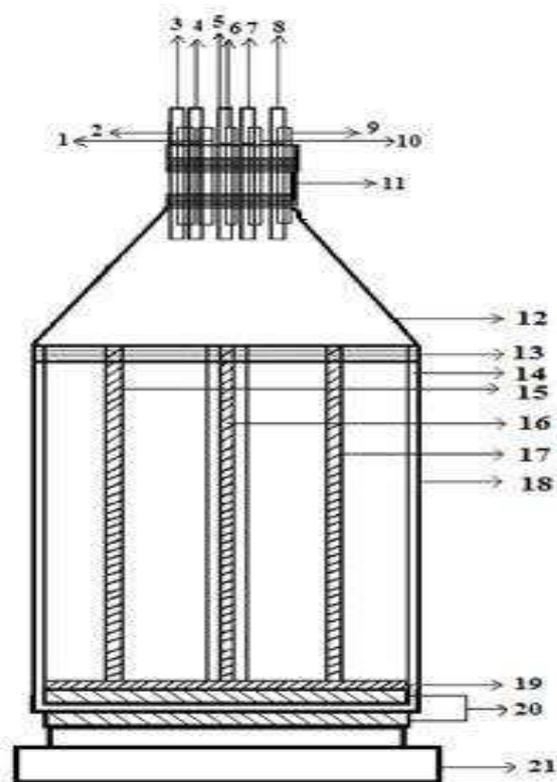
#### 2.3.1. Assay of antimicrobial compound

Determination of antimicrobial activity was carried out following the method described in [10]

by centrifuging the liquid medium containing the suspended cells and plating 150–200  $\mu\text{L}$  of the supernatant against *Staphylococcus aureus* MTCC 96 as the test microorganism. Maximum antimicrobial activity at a defined time interval is described as peak antimicrobial activity (PAMA) corresponding to the diameter of zone of inhibition.

### 2.3.2. Measurement of biofilm formation

Biofilm formation was quantified by scraping of an area of approximately 850  $\text{cm}^2$  area of the total biofilm surface with a sharp scalpel at the end of the experiment and measuring the dry weight on an electronic digital balance (AFCOSET, Model ER-180A, Mumbai, India) as described [11].



**Fig. 1a** Schematic of the the ‘extended surface biofilm reactor (ESBR)’. Details of the individual parts are: 1- Port for air in, 2- Sampling port, 3- Temperature sensor, 4-Antifoam port, 5-Inoculation and medium addition port, 6-Acid port, 7-pH sensor, 8-Alkali port, 9-DO sensor, 10- Port for air out, 11- Neck, 12- Funnel portion, 13- Threading, 14- Inner configuration,

15, 16, 17-Rectangular strips of inner configuration, 18- Lower cylindrical portion, 19-Circular disk, 20- Magnetic coupling, 21-Motor.



**Fig. 1b** Extended surface biofilm reactor (ESBR)



**Fig. 2** Inner configuration of the Extended surface biofilm reactor (ESBR)

## 3. RESULTS AND DISCUSSION

### 3.1. Small-scale Culture Studies in 420 mL Polymethylmethacrylate Conico-cylindrical Flask (PMMA-CCF) to Investigate the Effect of pH and Temperature on Antimicrobials Production by *Streptomyces sundarbansensis* sp. nov.

It is well known that optimum growth temperature for *Streptomyces* strains is around 30°C. Therefore, in order to determine the pH range to

be studied, 420 mL polymethylmethacrylate conico-cylindrical flask cultures (containing 100 mL medium) of MS1/7<sup>T</sup> at 30 °C were initially considered at pH 5, 7, and 9, respectively. Antimicrobials production was maximum in alkaline pH (PAMA of 28 mm) compared to the neutral (PAMA of 26 mm) and acidic pH (PAMA of 24 mm). Based on these preliminary findings, the pH range to be examined further was selected as 7~10 and temperature range as 30 ± 5°C for further detail study. Accordingly a 4 × 3 experimental design was performed to examine all combinations of 4 pH values (i.e. 7, 8, 9, and 10) and 3 temperature values (i.e. 25, 30, and 35°C). Fig. 3 shows the time course of antimicrobials production by MS1/7<sup>T</sup> in PMMA-CCF cultures for different pH values at 30°C (two other sets of such time profiles at different pH values, respectively for 25 and 35°C are not shown here for the sake of brevity - the summarized data of PAMA for these conditions are given in Table 1). At all the pH values, the antimicrobial activity curves in Fig. 3 are seen to rise steadily up to a peak, thereafter falling off. With increase in pH, the corresponding PAMA also increases - being 26 mm at pH 7, 27 mm at pH 8, 30 mm at pH 9, and 41 mm at pH 10. In fact, over the pH range 7~10, at 30°C, there is steady increase in PAMA from 26 to 41 mm. From Table 1, PAMA is found to be highest at 30°C, pH 10. Biofilm formation is also highest at 30°C expressed in dry cell weight 0.3896±0.0028g compared to 35°C (0.1024±0.0008g) and 25°C(0.0932±0.0009g).

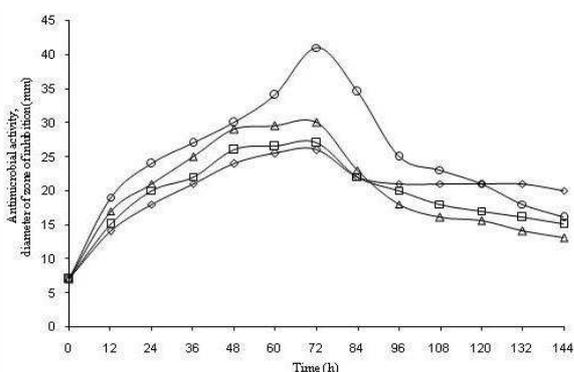


Fig. 3 Time course of antimicrobials production by the

marine isolate *Streptomyces sundarbansensis* sp nov. in PMMA-CCF cultures at temperature 30°C and different pH conditions: ◊ - 7, ◻ - 8, △ - 9, ○ - 10. (Values of diameter of zone of inhibition, include cup diameter of 7 mm).

Temperature			
25°C	30°C		35°C
pH	PAMA(mm)	PAMA(mm)	PAMA(mm)
7	14	26	18
8	16	27	20
9	17	30	22
<b>10</b>	21	<b>41</b>	24

Table 1. Effect of pH and temperature on peak antimicrobial activity (PAMA, reported as diameter of zone of inhibition, mm), during the production of antimicrobials by intertidal actinobacteria *Streptomyces sundarbansensis* sp. nov. in 420 mL Polymethylmethacrylate Conico-cylindrical Flask (PMMA-CCF)

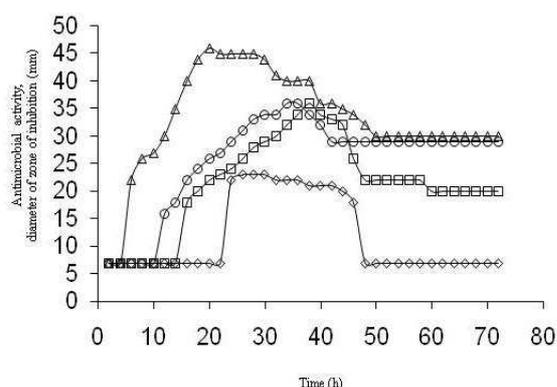
### 3.2. Preliminary Experimental Studies in ESBR

Due to the fact that in PMMA-CCF culture studies, as PAMA increased steadily in the alkaline pH range (at 30°C) with increase in pH up to pH 10, it is important to determine whether this increasing trend continues beyond pH 10 or not. Therefore, a set of experiments were performed in the ESBR operated at 30°C and 50% submergence conditions with maximum permissible aeration (i.e. 0.75 L/min), with initial medium pH varying from 8 to 11. The resulting time profiles for antimicrobial activity are shown in Fig. 4.

The time profiles of antimicrobial activity in Fig. 4 are described by the characteristic that once the peak antimicrobial activity is reached, it is maintained over a plateau for a few h, before declining. The PAMA is observed to steadily increase in the pH range 8~10 (from 36 mm at pH 8 to 46 mm at pH 10), but on further increase of pH to 11, PAMA drastically reduces to 23 mm. The time to attain the peak also decreases in the pH range 8~10, from 38 to 20 h, but again increases to 26 h for pH 11. Thus PAMA steadily increase with increasing pH up to pH 10, but fall

on further increase of pH to 11, thereby showing a clear peak at pH 10 for PAMA. Biofilm formation is also highest at pH 10 expressed in dry cell weight  $6.632 \pm 0.0048$ g compared to pH 8 ( $3.493 \pm 0.0029$ g), pH 9 ( $4.763 \pm 0.0038$ g) and pH 11 ( $1.932 \pm 0.0016$ g).

Thus, for the ESBR with maximum permissible aeration (here, 0.75 L/min) and cultivation temperature at 30°C, antimicrobials production is evidently maximized at initial pH 10, evaluated in terms of PAMA. Therefore, based on these results, ESBR studies were further performed by studying the effect of aeration and submergence level of the inner configuration, at temperature 30°C and initial pH 10.

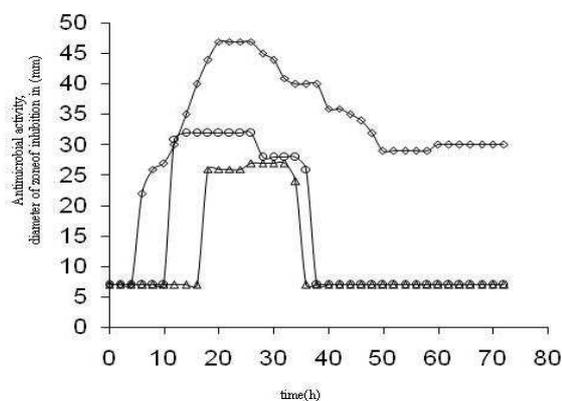


**Fig. 4** Effect of initial medium pH ( $\square$  - 8,  $\circ$  - 9,  $\triangle$  - 10,  $\diamond$  - 11) on antimicrobials production by the marine isolate *Streptomyces sundarbansensis* sp nov. in ESBR (i.e. 50% submergence and rotational speed 140 rotation per minute) with maximum permissible aeration (i.e. 0.75 L/min) at temperature 30°C. (Values of diameter of zone of inhibition, include cup diameter of 7 mm).

### 3.3. Designed Experimental Study in ESBR

The submergence level was varied from 50% by  $\pm 25\%$  at rotational speed maintained at 140 rpm and also the aeration rate lowered in a stepwise manner from its maximum permissible value (with the given experimental setup) of 0.75L/min that would result in different set of operating conditions. Therefore, to investigate simultaneously the effect of aeration rate and inner

configuration submergence level on antimicrobials production in ESBR, a 3<sup>n</sup> experimental design may be adopted, wherein each of two ( $n = 2$ ) parameters viz. aeration and inner configuration submergence are considered at 3 levels viz. high, medium, and low: 0.75, 0.50, and 0.25 L/min for aeration rate and 75, 50, and 25% for inner configuration submergence level. All ESBR cultivations were carried out at the optimum temperature and initial pH values determined with small-scale PMMA-CCF cultures (i.e. 30°C and pH 10). Each ESBR experiment was replicated at least twice and averages of the values with less than 10% deviation are reported. In Fig. 5 the effect of aeration on antimicrobials production is shown with the ESBR operated at 50% submergence condition. As in Fig. 4, for all the antimicrobial activity profiles shown, it is observed that once the peak is attained, it is maintained over a plateau for a few h (between 6~12 h), before declining. With increase in aeration from 0.25 to 0.75 L/min, PAMA steadily increases from 27 to 47 mm, the time to attain the peak decreases steadily from 26 h to 20 h. Biofilm formation is also highest at aeration level 0.75 L/min expressed in dry cell weight  $6.931 \pm 0.0058$ g compared to 0.50 L/min ( $2.923 \pm 0.0018$ g) and 0.25 L/min ( $1.672 \pm 0.0008$ g). Evidently, adequate aeration is crucial for antimicrobials production in ESBR by the marine isolate *Streptomyces sundarbansensis* sp nov.



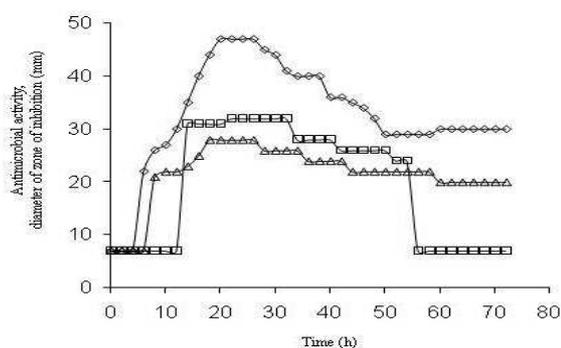
**Fig. 5** Effect of aeration ( $\Delta$  - 0.25 L/min,  $\circ$  - 0.50

L/min,  $\diamond$  - 0.75 L/min) on antimicrobials production by the marine isolate *Streptomyces sundarbansensis* sp nov. in ESR (i.e. 50% submergence and rotational speed 140 rotation per minute) at temperature 30°C and initial medium pH 10. (Values of diameter of zone of inhibition, include cup diameter of 7 mm).

With aeration at the maximum permissible level, it may be seen from Fig. 6 that the PAMA value of 47 mm for the 50% submergence is clearly higher than the values obtained for 25% (28 mm) and 75% (32 mm) inner configuration submergence. Biofilm formation is also highest at submergence level 50% expressed in dry cell weight  $6.948 \pm 0.0059\text{g}$  compared to 75% ( $3.423 \pm 0.0024\text{g}$ ) and 25% ( $1.972 \pm 0.0009\text{g}$ ). The ESR data on antimicrobials production by MS1/7<sup>T</sup> summarized above in Table 2, confirms that the 50% inner configuration submergence condition with maximum permissible aeration certainly gives highest values for PAMA as well as maximum biofilm formation (Fig. 7).

Again, at all submergence levels examined PAMA increase with increasing aeration. Particularly, at 50% submergence, increasing aeration from 0.25 to 0.75 L/min causes PAMA to increase by 74%. The maximum values of PAMA obtained in the ESR experiments are evidently much higher than the corresponding best values in the 420 mL PMMA-CCF experiments - PAMA of 47 mm compared to 41, an increase of 15 % in PMMA values. It is this tremendous increase in antimicrobials production in a novel ESR, which is the motivating factor for using these novel bioreactors for cultivation of antimicrobial-producer marine microbes.

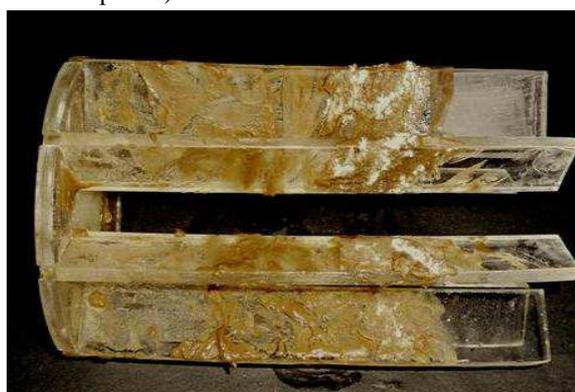
Overall, the ESR may be considered as the preferred alternative to the STBR (stirred tank bioreactor) for production of novel metabolites from estuarine microorganisms for its much higher surface/volume ratio, presence of inner configuration that helps in biofilm formation, lower costs, and easy operability.



**Fig. 6** Effect of inner configuration submergence level ( $\diamond$  -50%,  $\square$  - 75%,  $\Delta$  - 25%) on antimicrobials production by the marine isolate *Streptomyces sundarbansensis* sp nov. in ESR (140 rpm, temperature 30°C, initial medium pH 10) with maximum permissible aeration (i.e. 0.75 L/min). (Values of diameter of zone of inhibition, include cup diameter of 7 mm)

Aeration rate (L/min)	Inner Configuration Submergence level		
	25%	50%	75%
	PAMA (mm)	PAMA (mm)	PAMA (mm)
0.25	23	27	24
0.50	26	32	30
0.75	28	<b>47</b>	32

**Table 2:**Effect of inner configuration submergence level and aeration rate on peak antimicrobial activity (PAMA, reported as diameter of zone of inhibition, mm), during the production of antimicrobials by intertidal actinobacteria *Streptomyces sundarbansensis* sp. nov. in an ESR (140 rpm, temperature 30°C, initial medium pH 10).



**Fig. 7** Surface-attached growth of *Streptomyces sundarbansensis* sp. nov. observed in the ESR at the end of the process.

#### 4. CONCLUSIONS

A novel ESBR with higher internal surface area was developed and shown to provide increased antimicrobials production by biofilm-forming actinobacteria *Streptomyces sundarbansensis* sp. nov. The significant enhancement in antimicrobials production by *Streptomyces sundarbansensis* sp. nov. in the ESBR may be attributed to the fact that the ESBR facilitates formation of microbial biofilm by providing substantial surface area (per unit volume of culture) for its attachment and growth, thus associated with antimicrobials production. For further insight into the functioning of this novel reactor, future study should aim to identify the origin of the antimicrobial activity by exploring the relationship between suspended cells and those in biofilm in terms of growth and antimicrobials production.

#### ACKNOWLEDGEMENTS

CSIR Senior Research Associateship (8584-A/2012-Pool) to S. Sarkar is thankfully acknowledged.

#### REFERENCES

1. Arumugam, M., A. Mitra, A. Pramanik, M. Saha, R. Gachhui, and J. Mukherjee (2011) *Streptomyces sundarbansensis* sp. nov., a novel actinomycete that produces 2-allyloxyphenol. *Int. J. Syst. Evol. Microbiol.* 61:2664-2669.
2. Arumugam, M., A. Mitra, P. Jaisankar, S. Dasgupta, T. Sen, R. Gachhui, U.K. Mukhopadhyay, and J. Mukherjee (2010) Isolation of an unusual metabolite, 2-allyloxyphenol from a marine actinobacterium, its biological activities and applications. *Appl. Microbiol. Biotechnol.* 86:109-117.
3. Decho, A.W. (2000) Microbial biofilms in intertidal systems: An overview. *Cont. Shelf Res.* 20: 1257-1273.
4. Marwick, J.D., P.C. Wright, and J.G. Burgess (1999) Bioprocess intensification for production of novel marine bacterial antibiotics through bioreactor operation and design. *Mar. Biotechnol.* 1:495-507.
5. Ortega-Morales, B.O., M.J. Chan-Bacab, S.D.C. De la Rosa-García, and J.C. Camacho-Chab (2010) Valuable processes and products from marine intertidal microbial communities. *Curr. Opin. Biotechnol.* 21:346-352.
6. Penesyan, A., S. Kjelleberg, and S. Egan (2010) Development of novel drugs from marine surface associated microorganisms. *Mar. Drugs* 8:438-459.
7. Pomponi, S.A. (1999) The bioprocess-technological potential of the sea. *J. Biotechnol.* 70:5-13.
8. Saha, M., P. Jaisankar, S. Das, K.K. Sarkar, S. Roy, S.E. Besra, J.R. Vedasiromani, D. Ghosh, B. Sana, and J. Mukherjee (2006) Production and purification of a bioactive substance inhibiting multiple drug resistant bacteria and human leukemia cells from a salt-tolerant marine actinobacterium isolated from the Bay of Bengal. *Biotechnol. Lett.* 28:1083-1088.
9. Sarkar, S., (2014) Enhanced antimicrobials and esterase production associated to biofilm formation by two estuarine isolates in a novel polymethylmethacrylate conicocylindrical flask. *Int. J. of Adv. Biotechnol. and Res.* 5: 242-261.
10. Sarkar, S., D. Roy, and J. Mukherjee (2010) Production of a potentially novel antimicrobial compound by a biofilm-forming marine *Streptomyces* sp. in a niche-mimic rotating disk bioreactor. *Bioprocess Biosyst. Eng.* 33:207-217.
11. Sarkar, S., D. Roy, and J. Mukherjee (2011) Enhanced protease production in a polymethylmethacrylate conico-cylindrical flask by two biofilm-forming bacteria. *Bioresour. Technol.* 102:1849-1855.
12. Sarkar, S., D. Roy, and J. Mukherjee, U.S. patent application US2012/0295293A1 (allowed by the USPTO)
13. Sarkar, S., J. Mukherjee, and D. Roy (2009) Antibiotic production by a marine isolate (MS310) in an ultra-low-speed rotating disk bioreactor. *Biotechnol. Bioprocess Eng.* 14:775-780.
14. Sarkar, S., M. Saha, D. Roy, P. Jaisankar, S. Das, L.G. Roy, R. Gachhui, T. Sen, and J. Mukherjee (2008) Enhanced production of antimicrobial compounds by three salt tolerant actinobacterial strains isolated from the Sundarbans in a niche-mimic bioreactor. *Mar. Biotechnol.* 10:518-526.

15. Yan, L., K.G. Boyd, and J.G. Burgess (2002) Surface attachment induced production of antimicrobial compounds by marine epiphytic bacteria using modified roller bottle cultivation. *Mar. Biotechnol.* 4:356–366.
16. Yan, L., K.G. Boyd, D.R. Adams, and J.G. Burgess (2003) Biofilm-specific cross-species induction of antimicrobial compounds in Bacilli. *Appl. Environ. Microbiol.* 69:3719–3727.