Int. J. Chem. Sci.: 8(3), 2010, 1553-1566

USE OF LUMINOUS BACTERIA AS A BIOSENSOR FOR MONITORING OF HEAVY METALS IN MARINE WATER

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ABSTRACT

Luminous bacteria were isolated from marine fishes like *sardinella longiceps*, those of characterized different species of luminous bacteria such as *Vibrio fisheri, V.harveyi,* and *photobacterium leiognathi*. The optimum growths of this species were performed under various growth conditions of different salt (NaCl) concentrations, pH and temperatures. The complex sea water medium exhibit the best result for the growth of luminous bacteria. The 3% NaCl (actual concentration in the medium), 37˚C (room temperature), pH – 7.2 (actual medium pH) are optimum conditions for the growth of luminous bacteria. From heavy metal toxicity analysis, it was observed that zinc induces the growth of luminous bacteria where as the cadmium inhibited the growth. Obviously, this investigation has concluded that luminous bacteria act as a biosensor for the detection of heavy metal pollution in marine water.

Key words: Luminous bacteria, Heavy metals, Marine water, Biosensor.

INTRODUCTION

The ecology of the luminous bacteria is complex[1-3]; even a given species can exhibit a variety of life-styles and inhabit several niches, including planktonic, saprophytic, symbiotic, and parasitic niches. Neither the dynamics of the bacterial populations nor the importance of the different niches to the overall ecology of the luminous bacteria is understood, even though a great deal of information is available concerning the distribution

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and abundance of various luminous species[3]. *V. harveyi* is one of the 11 *Vibrio* species reported to be infecting cultured penaeid shrimps in Asia. It is believed to be the most dominant species of luminescent *Vibrio* present in shrimp ponds in the Philippines with an incidence rate of 65.5%. Living organisms require trace amounts of some heavy metals, including iron, cobalt, copper, manganese, molybdenum, vanadium, strontium and zinc but excessive levels can detrimental to the organism[4]. Other heavy metals such as mercury, lead and cadmium are toxic metals. They have no known vital or beneficial effect on organisms and their accumulation over time in the bodies of mammals can cause serious illness. The pathway for toxic effects of humans is normally,

- For the entry of heavy metals into the atmosphere as individual stack gas.
- To enter the soil as soil contaminant.
- To enter the ground water as water pollutant.
- To be deposited in ocean bottoms or bay mud, which are the materials at a later time to be dredged to the surface.

In medical usage, the definition is considerably looser and includes all toxic metals irrespective of their atomic weight. “Heavy metal poisoning” can include excessive amounts of iron, manganese, aluminum or beryllium or such a semi-metals as arsenic as well as the true heavy metals. It paradoxically excludes bismuth, the heaviest of stable elements because of its non-toxicity.

Most of the materials, which reach the sea, disintegrate either through simple chemical reactions or because of the activities of bacteria and some larger organisms. There are some substances nevertheless, which are either extremely stable or else, they have a very slow rate of degradation. The organic compounds in domestic sewage waste from the food industry and agricultural fertilizers all belong to the first category while plastics, heavy metals and nuclear wastes belong to the second. Heavy metals are not as visible as the types of pollution, we have considered so far, but from their position hidden within the sediments, must be considered as a very serious marine pollution hazard. The term "heavy metals" is used to denote elements with specific weight higher than those of iron (Fe) and mainly lead (Pb), mercury (Hg), copper (Cu), cadmium (Cd) and chromium (Cr)[5,6]. Metals have many sources from which they can flow into the water body. These sources are from industrial, domestic waste water, agricultural discharge, and atmospheric pollution, which affects the food chain and aquatic environments eventually causing extinction of aquatic animals and degrading the quality of human life. The main focus of this study was to isolate, identify the

luminous bacteria, characterize the growth medium and test the effect of heavy metals on the growth of luminous bacteria for the detection of heavy metal pollution in marine water.

EXPERIMENTAL

Materials and methods

Materials (Saginella) were collected from Tuticorin coastal and Rameshwaram coastal for the isolation of luminous bacteria. Fortnightly collections of shrimp samples were made to study the distribution of luminous bacteria from the shrimp culture ponds from Tuticorin and Rameshwaram. To isolate and characterize the luminous bacteria in particular and fish species, the collected shrimp samples were transferred immediately to estuary or pond water filled polythene bags. Continuous aeration was provided using battery operated air pump. Various stages of larvae of *P. monodon* and *P. indicus* were maintained separately in sea water recirculatory system with water of salinity 20 + ppt. The shrimp were fed *ad libitum* with shrimp feed supplied by the hatcheries. To isolate luminous bacteria, complex sea water (SWC) medium with 3 mL glycerol per litre and adjusted to a pH of 7.2 was used as suggested by Hastings and Nealson[7]. For the preparation of solid medium, 15 g of agar was added to 1 litre of the medium.

Isolation of luminous bacteria

For the isolation of free living luminous bacteria from estuary, and shrimp culture ponds, water samples were serially diluted separately and 0.1 mL of the sample was inoculated on pre-poured SWC agar plates and incubated at room temperature (28 ± 2°C). The sediment samples were also serially diluted and plated accordingly. After incubation for 24 h, the colonies were observed in dark and morphologically dissimilar colonies were isolated at random and subsequently sub-cultured repeatedly on SWC agar for purity. They were maintained in SWC agar slants for further characterization. Isolation procedure was similar to the procedure adopted for the isolation of luminous bacteria. All the samples were plated on pre-poured Tryptic Soy Agar (TSA) with 75 % aged seawater.

Identification and quantification of luminous bacteria

To identify luminous bacterial isolates up to species level, the schemes provided[8-10] were followed. The identification chart is given in Table 1. Seasonal distribution of luminous bacteria was quantified from water, sediment and shrimps from estuary and culture ponds. The density of luminous bacterial count (LBC) in water was quantified as CFU mL^{-1}, from sediment and gut CFU g^{-1} and from exoskeleton and gill as cfu/cm^2. Seasonal distributions of total heterotrophic bacteria were quantified from water, sediment and

shrimps of estuary and culture ponds. The density of total viable bacterial count (TVC) in water were quantified as cfu mL^{-1}, from sediment and gut cfu g^{-1} and from exoskeleton and gill were cfu/cm^2. The growth of luminous bacteria was studied using different pH conditions like acidic, basic and neutral conditions. The luminous bacterial growth for different heavy metal concentrations were studied using standard plate method by this method. The number of bacterial colonies were calculated for different heavy metal concentrations. From this method, the intensity of the luminescence were studied.

Table 1: Identification scheme for different species of luminous bacteria

Tests	*V. harveyi*	*V. fischeri*	*P. leiognathi*
Gram's staining	√	√	√
Luminescence pattern	√	√	√
Growth at different NaCl concentration in the medium			
1%	√	√	√
3%	√	√	√
6%	√	√	√
8%	√	√	√
10%	√	√	√
Enzyme analysis			
Amylase	√	√	√
Lipase	√	√	√

RESULTS AND DISCUSSION

In the present study, the light output of the luminous bacteria has been studied to monitor the heavy metals, most of which reach the marine environment through one or the other sources. The different species of luminous bacteria were isolated from different parts (such as gill and gut) of the marine fishes. The isolated luminous bacteria were used for further analysis such as identification of the species, characteristic studies, monitoring of heavy metals such as zinc and cadmium. The bioluminescence of the bioluminescent bacteria was caused by transcription induced by population-dependent quorum sensing. The luminescence is only seen, when population density reaches a certain level. The

luminescence appears to follow a circadian rhythm, that is, it is brighter during the night times than day time. The population size of the luminous micro flora was more in the gut of the gastropods, when compared to that in the water and the sediment. Maximum growth of these luminous bacteria was observed at 2-5 % NaCl concentration depending on the organism from which the bacteria was isolated. The effects of different NaCl concentration (Table 2, Fig. 1), temperature (Table 3, Fig. 2) and pH (Table 4, Fig. 3) on the growth of the luminous bacteria were studied.

The slackness of growth below pH 7 and above pH 8 and peak growth encountered at pH 7.2 (Table 4) suggests that these luminous bacteria prefer neutral pH. All the luminous isolates favoured an optimum temperature of 37˚C (Table 3). pH affects bacteria the same way, it affects all living things. Extremes of pH affect the function of enzyme systems by denaturing them. However, bacteria become adapted over time to their surroundings. It depends on the bacteria and what its natural environment is ? Bacteria that are isolated from marine fishes are generally adapted to a pH of about 7.4, which is slightly basic. The response of luminous bacterial cultures to conditions encountered in the fish gut such as neutral pH, the presence of bile salts, gastric juice and lysozyme was examined. The organisms preferred neutral pH. Bile salts did not inhibit their growth. Neither lysozyme nor gastric juice affected their growth and viability to any extent. The room temperature (37˚C) is the optimal for growth of luminous bacteria. So, no special equipment is needed (e.g. incubators) to provide optimum temperature for cultivation of bioluminescent bacteria. Data (in the Table 5, Fig. 4 and Table 6, Fig. 5) show the effect of zinc on the growth of the bioluminescent bacteria. This indicate that zinc acts as inducer and it helps in the growth of bacteria. There are several possible mechanisms described for the effect of zinc on the growth of the bacteria. It has been suggested that zinc binds to the membranes of microorganisms, and prolonging the lag phase of the growth cycle and increasing the generation time of the organisms so that it takes each organism more time to complete cell division[8].

Bioluminescence was observed at all temperatures that the organism grow that supported the growth of the bacteria. The effects of temperature on luminous bacteria revealed that the population of luminous bacteria decreased during cold weather; however, the bacterial population in the sediment persisted unaffected. The influence of some heavy metals on luminous bacteria was studied to monitor the heavy metal concentration in marine water and marine foods. Data (in Table 7, Fig. 6 and Table 8, Fig. 7) show the effect of cadmium on the growth of bioluminescent bacteria. From this experiment, it is clear that cadmium always suppressed the growth of bacteria. Because concentrations of the heavy

metals inhibiting the luminescence by 50% resulted in damaging effects upon bacteria. Due to the changes in their permeability, the cells had damage of membranes. The long action of these substances changed the membrane permeability resulting in increased sensitivity of bacterial luminescence to produce toxic substances to arrest the growth of bacteria. In this heavy metal treatment, the Zn metal had the inducer activity and the Cd metal had suppressor activity. The growth of the bacteria was increased in increasing Zn concentration at a particular time period (Tables 6 and 7). The growth of the bacteria was decreased in increasing Cd concentration and also the increasing the time of incubation (Tables 7 and 8).

Table 2: Comparative analysis of effect of NaCl on growth of bioluminescent bacteria collected from different parts of fish

Absorbance at 630 nm		
NaCl concentration	**Gill sample**	**Gut sample**
1%	0.068	0.007
2%	0.065	0.032
3%	0.070	0.026
4%	0.068	0.008
5%	0.065	0.015
6%	0.054	0.010
7%	0.067	0,011
8%	0.064	0.018
9%	0.061	0.017
10%	0.062	0.010
Control	0.066	0.015

Control medium contain 3% NaCl
In all treatment, the medium containing 3% of NaCl along with appropriate treatment concentration of NaCl.

Table 3: Comparative analysis of effect of different temperatures on different samples of bioluminescence bacteria

Absorbance at 630 nm		
Temperature	**Gill sample**	**Gut sample**
37˚C	0.140	0.141
45˚C	0.122	0.135

Table 4: Comparative analysis of effect of different pH on different samples of bioluminescence bacteria

Absorbance at 630 nm		
pH	**Gill sample**	**Gut sample**
5	0.133	0.120
7	0.147	0.118
9	0.138	0.141
Control (7.2)	0.148	0.132

Table 5: Growth of luminous bacteria at different zinc concentrations

Absorbance at 630 nm						
Time (hour)	**Zn concentration**					
	0.25%	**0.5%**	**0.75%**	**1%**	**Control with organism**	**Control without organism**
0	0.17	0.18	0.20	0.22	0.12	0.16
24	0.17	0.19	0.24	0.26	0.50	0.16
40	0.18	0.20	0.25	0.27	0.79	0.16
48	0.18	0.25	0.27	0.29	0.80	0.16
90	0.19	0.28	0.30	0.34	1.18	0.16

Table 6: Growth of luminous bacteria at different zinc concentrations

Absorbance at 630 nm					
Time (hour)	**Zn concentration**				
	2%	**3%**	**4%**	**5%**	**Control**
0	0.244	0.267	0.287	0.306	1.268
24	0.320	0.370	0.390	0.400	1.360
40	0.470	0.480	0.520	0.527	1.558
48	0.558	0.651	0.694	0.701	1.680
90	0.567	0.675	0.703	0.723	1.732

Table 7: Growth of luminous bacteria at different cadmium concentrations

Absorbance at 630 nm						
Time (hour)	**Cd concentrations**					
	0.25%	**0.5%**	**0.75%**	**1%**	**Control with organism**	**Control without organism**
0	0.148	0.146	0.142	0.14	0.12	0.02
24	0.142	0.135	0.133	0.122	0.5	0.02
40	0.139	0.132	0.124	0.118	0.79	0.02
48	0.133	0.128	0.12	0.1	0.8	0.02
90	0.126	0.122	0.117	0.07	1.18	0.02

Table 8: Growth of luminous bacteria at different cadmium concentrations

Absorbance at 630 nm					
Time (hour)	**Cd concentration**				
	2%	**3%**	**4%**	**5%**	**Control**
0	0.12	0.118	0.110	0.103	1.268
24	0.120	0.113	0.106	0.1	1.360

Cont…

40	0.115	0.11	0.102	0.098	1.558
48	0.096	0.093	0.087	0.076	1.680
90	0.068	0.063	0.059	0.053	1.732

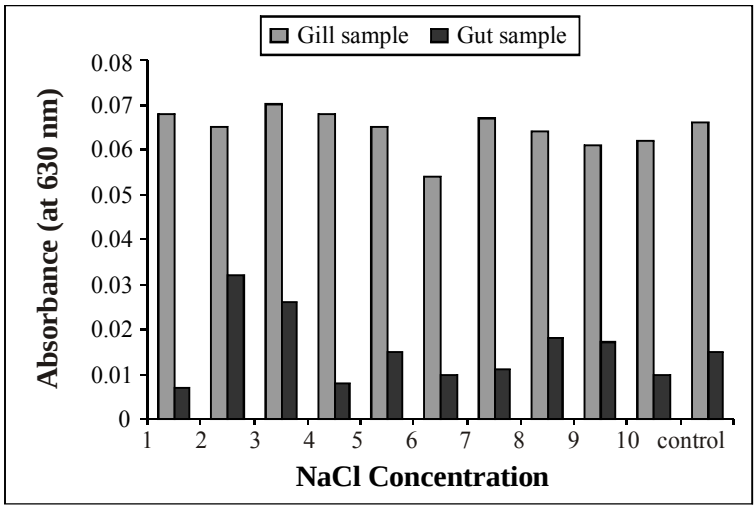

Fig. 1: Comparative analysis of effect of NaCl on growth of bioluminescent bacteria collected from different parts of fish

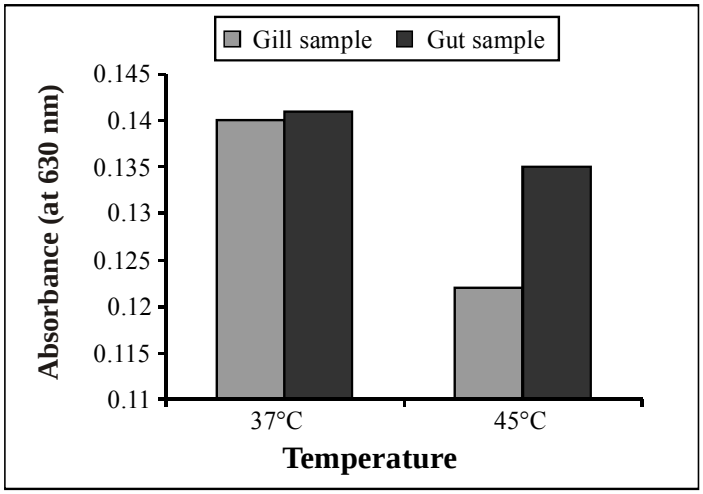

Fig. 2: Comparative analysis of effect of different temperatures on different samples of bioluminescence bacteria

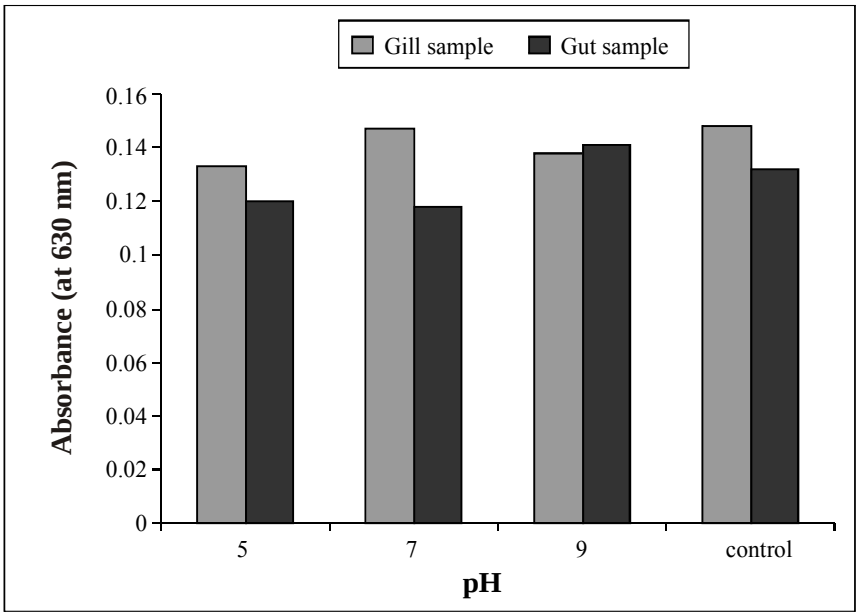

Fig. 3: Comparative analysis of effect of different pH on different samples of bioluminescence bacteria

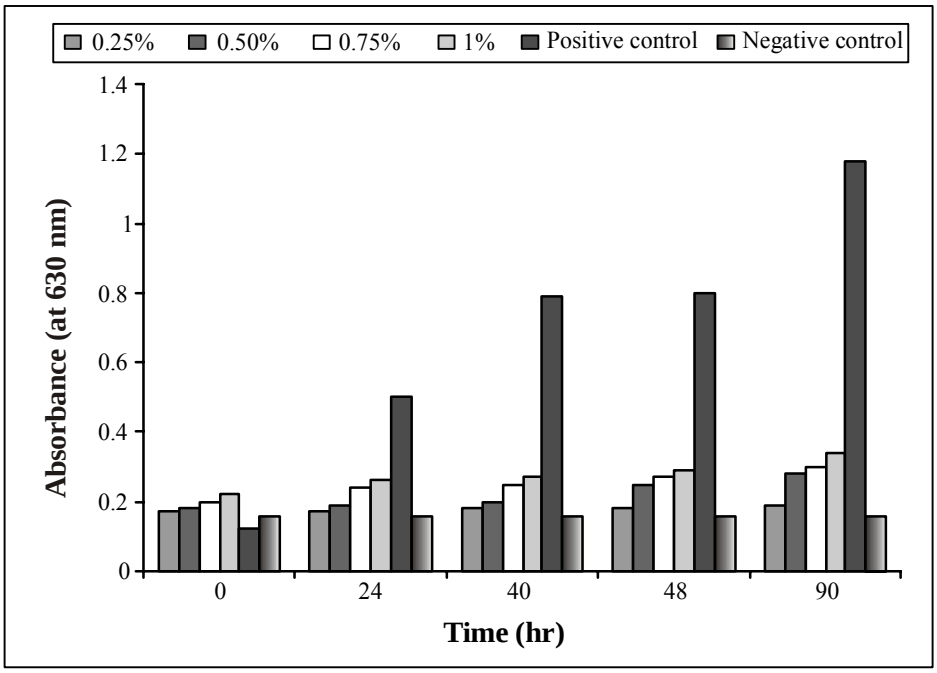

Fig. 4: Growth of luminous bacteria at different zinc concentrations

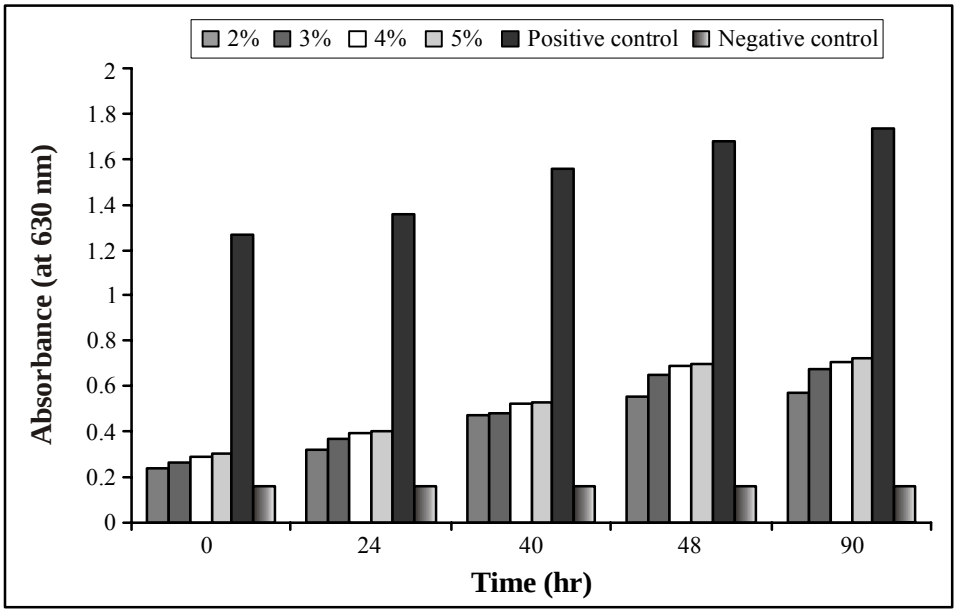

Fig. 5: Growth of luminous bacteria at different zinc concentrations

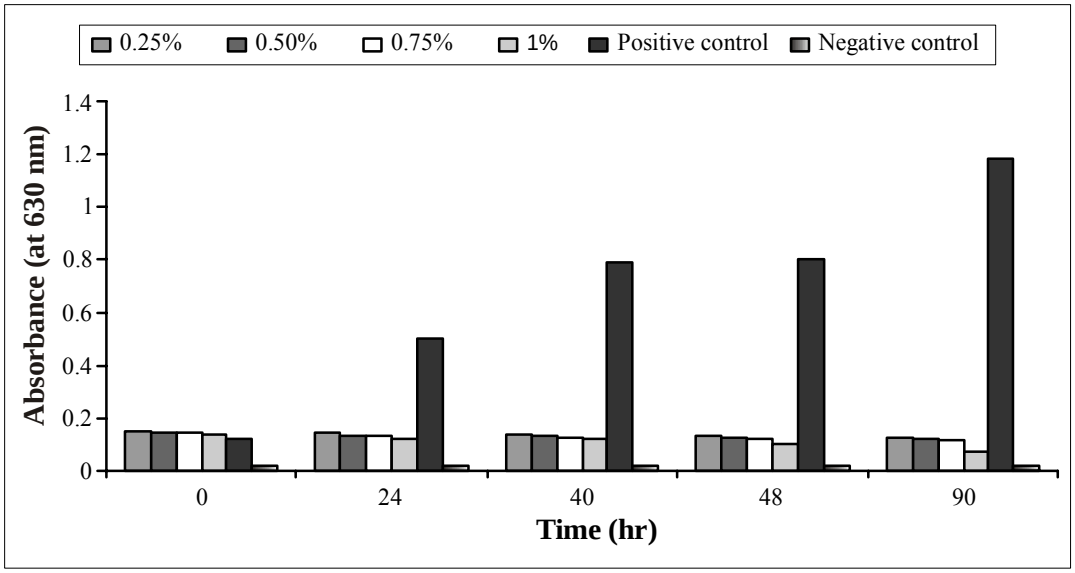

Fig. 6: Growth of luminous bacteria at different cadmium concentrations

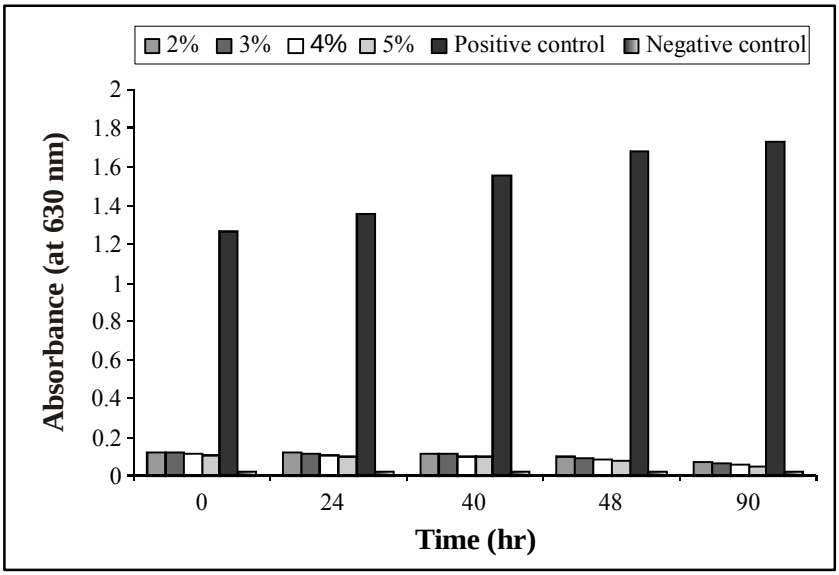

Fig. 7: Growth of luminous bacteria at different cadmium concentrations

CONCLUSION

From the overall studies, it is confirmed that the luminous bacteria act as a biosensor for the detection of heavy metal pollution. Heavy metals such as zinc and cadmium have influenced the growth of the bacteria. Zinc (inducer) includes the growth of luminous bacteria but cadmium (suppressor) inhibits the growth of bacteria. It will help to monitor the heavy metal pollution level in the sea and to take effort to reduce the heavy metal pollution in marine water by using luminous bacteria.

ACKNOWLEDGEMENT

The authors thank Shri. A. N. Radhakrishnan, Managing Trustee, Meenakshi Ammal Trust, for providing round the clock facilities, and Dr. S. Muthu, Principal, Arulmigu Meenakshi Amman College of Engineering, for his moral support and continuous encouragement.

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Revised : 13.02.2010 *Accepted :18.02.2010*