Novel microbial biosensor for assaying toxicity of waste water

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ABSTRACT

The contamination of the environment and its direct relation to human health has raised the standards pertaining to discharge of liquid effluents into water bodies. The dramatic increase in anthropogenic activity over the past decade has led to concerns about the sustenance of life on earth. Monitoring the pollution levels and making sustainable development is the only way towards maintaining stewardship of the environment.

It is important to have methodologies to assess the quality of water. So, we have developed a novel biosensor that can assess the efficiency of wastewater treatment plants. Biochemical Oxygen Demand (BOD), is one of the primary parameters used to determine the level of toxicity of waste water and is defined as the amount of dissolved oxygen needed by aerobic biological organisms to metabolise the organic material present in the sample. The key sensing element of our device is the recombinant *E. coli* strain containing the lux genes A-E, which encodes for the luciferin-luciferase system. The genes are kept under the control of an arabinose based switch and characterised for optimal concentration of arabinose. The carbon source is metabolised via the glycolysis-TCA pathway to generate ATP and NADPH which regenerate the substrates required for the above operon. The presence of biodegradable organic pollutants would lead to bioluminescence and this would be correlated to the conventional BOD_5 values.

In order to improve the functionality of the device viz. stability and reusability, the genetically engineered microbes have been immobilised in sodium alginate beads. This immobilised cell strategy would lead to increased stability as compared to the existing O_2 electrode based sensing devices.

Keywords: ***microbial biosensor; BOD; luciferase; toxicity***

1. INTRODUCTION

The unprecedented economic growth over the past decade has led to environmental degradation. Water pollution is one such global problem and may soon lead to catastrophic effects on the entire biosphere if left unaddressed. A variety of indicators are used to assess the quality of waste water – common physical parameters like total suspended solids (TSS), turbidity to widely used parameters like biological oxygen demand (BOD). The efficiency of waste water treatment plants is usually measured in terms of BOD as it quantifies the biologically degradable pollutants unlike other organic matter based measures like total organic carbon (TOC) and chemical oxygen demand (COD).
BOD refers to the amount of dissolved oxygen required to metabolise the organic pollutants in the sample by aerobic microbes. The conventional method of estimating BOD involves monitoring the levels of dissolved oxygen at 20 ^{0}C in dark room over a period of 5 days which

is time consuming and quite cumbersome owing to the stringent conditions. Although several biosensors have been developed based on the principle of Clark's oxygen electrode, the membrane immobilised microbes loose activity over time [1].

An ideal biosensor would have the ability to be used in an *in-situ* fashion, reusable over a long period of time and exhibit fast response kinetics. In this study, we attempt to develop a small scale biosensor that would possess the above salient features.

2. WORK DESCRIPTION

2.1 Choice of plasmid & transformation

Plasmids BBa_K325909 and BBa_K769020 containing *lux A-E* genes of *V. fischeri* were obtained from iGEM 2012 kit plate. The latter has the genes encoding for lumazine protein (*LumP*) fused downstream to the *lux* operon. The genes in both of these plasmids are under the control of arabinose based promoter (P_{bad}). Both the plasmids were transformed into *E. coli* DH5α strain. The successful transformants were screened by picking colonies grown on LB agar plates containing chloramphenicol (25µg/ml) incubated overnight at 37 ^{0}C.

2.2 Cell culture preparation

LB media was inoculated with a single transformed colony and grown at 37 ^{0}C, 180 rpm for 9h. A certain volume of primary culture was added to fresh LB media so as to have an initial OD_{600} of 0.2 and the volume was made to 50ml. The cells were harvested at the onset of stationary phase (OD_{600} = 0.8). For induction, 0.25% (w/v) of L-arabinose was added and incubated at 20 ^{0}C, 180 rpm. The bioluminescence was measured at regular intervals using the luminometer (Smart Line TL, Titertek-Berthold Co.) by aliquoting 200µl of the culture into the 5ml vial.
The effect of L-arabinose concentration and temperature were studied by inducing the aliquots of harvested cells at different concentrations of L-arabinose and by incubating at different temperature respectively.

2.3 Immobilization

In order to increase the stability and reusability of the cells, it was decided to entrap the bacteria into sodium alginate beads. Upon harvesting the cells followed by arabinose induction, the culture was centrifuged at 2500rpm for 10 minutes. The supernatant was discarded and the pellet was dissolved in 3 ml of 3% (w/v) sodium alginate solution. This solution was gently poured through a 5ml syringe into 50 ml of 2% calcium chloride solution leading to instantaneous formation of beads entrapped with the bacteria. The beads were washed with LB media twice and then stored in phosphate buffer saline (PBS) at 4 ^{0}C for later usage.

2.4 Calibration of BOD values

The sodium alginate beads having the cells were incubated at different concentrations of Glucose-Glutamic Acid (GGA) solution under the optimal temperature and arabinose concentration, as determined by previous set of experiments. One bead per well was taken in a series (in a 96 well microplate). 200 µl of 0 ppm, 3 ppm, 6 ppm, 9 ppm, 12 ppm, 15 ppm and 50 ppm of GGA solution was added to the wells respectively. 200 µl of LB media containing

the bead was used as a positive control. 0.25% L-arabinose was added and incubated for 5hrs. The bioluminescence was observed using a Bio-Rad ChemiDoc Imager.

3. RESULT & DISCUSSION

3.1 Enhancing the bioluminescence

The luminous intensity of the light emitted by the recombinant *E. coli* containing two different plasmids viz. BBa_K325909 (having genes encoding *lux A-E* only) and BBa_K769020 (having genes encoding *lux A-E and LumP*) were compared to choose the best system for our microbial biosensor (Fig. 2). It is known that the presence of lumazine protein (*LumP*) in *Photobacterium* species leads to a dip in wavelength of light emitted from 495nm to 475nm. The plasmids were isolated and the presence of insert was confirmed by restriction digestion assay followed by gel electrophoresis (Fig 1a). The plasmids were transformed into an bacterial expression system for the production of luminescence. The luminous intensity of *lux/LumP* system was observed to be more as compared to the normal lux system. Figure 1b depicts the variation of luminous intensity with time for both the systems.

(a) (b)

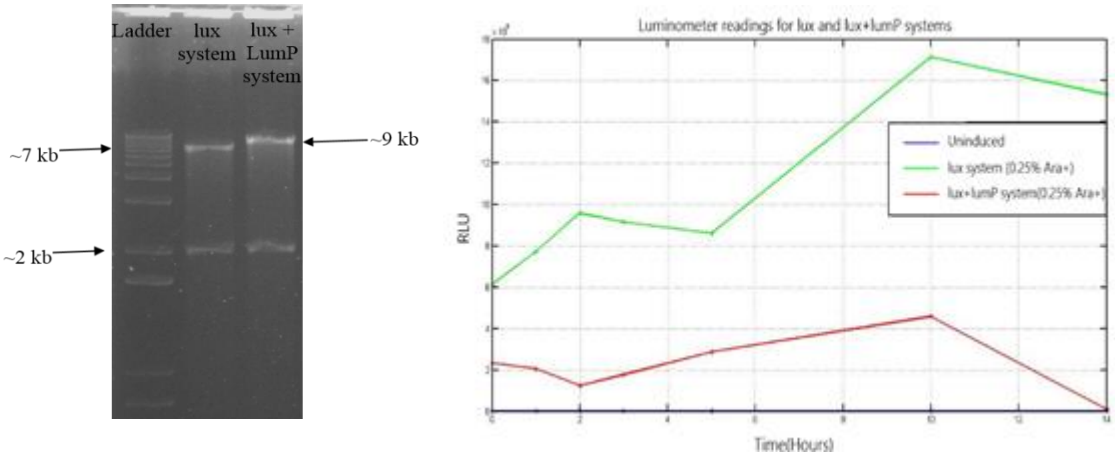

Fig.1. (a) confirmation of DNA parts in the biobricks via restriction enzyme assay (b) Comparison of luminescence intensity for lux and lux/LumP system

3.2 Studying the effect of L-arabinose concentration

The genes encoding for *lux* operon in our biosensor are under the control of pBAD promoter. Hence, the presence of arabinose in medium is necessary for transcription of these genes and eventually emitting light. As per the literature iGEM team Tokyo-NoKoGen], it's reported that 10mM and 16.33mM (0.25% w/v) of arabinose is best suited for maximising the amount of bioluminescence. We compared the expression of *lux* operon along with a negative control and found that the luminous intensity was higher at 0.25% (w/v) of L-arabinose as shown in Fig.3.

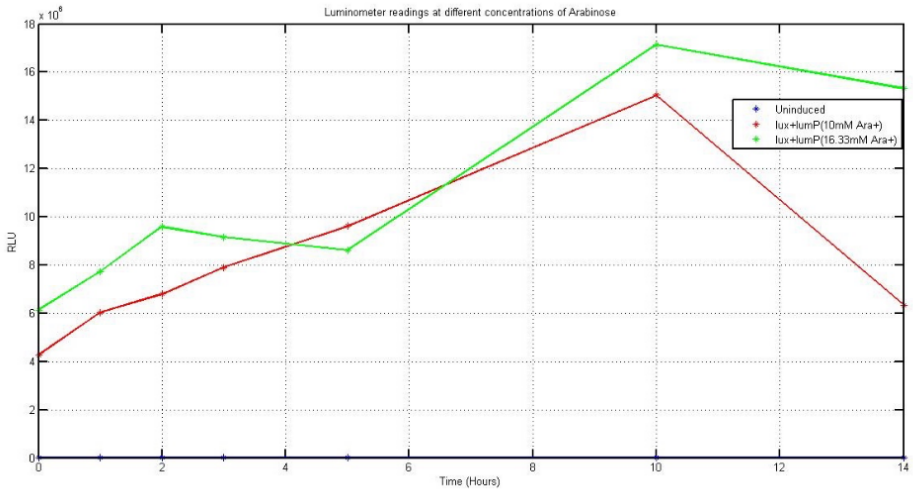

Fig.3. Comparison of luminescence intensity for *lux* vs *lux/LumP* system

3.3 Optimization of temperature

In order to make a stable system capable of producing maximum luminescence for longer time, we thought of optimizing the temperature conditions. Here, we studied the effect of three different temperatures viz. 20 °C, 25 °C and 37 °C. From Fig 4, it can be inferred that the luminescence is sustained for longer time when incubated at 20 °C and thus we chose this temperature for further experiments.

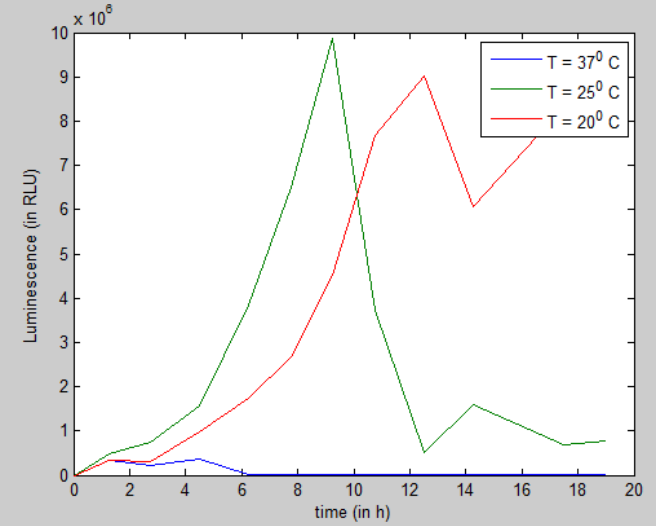

Fig.4. Comparison of sustenance of bioluminescence across different incubation temperatures

3.4 Immobilisation & calibration

The use of sodium alginate beads for immobilisation of bioluminescent bacteria provides increased stability/life, in comparison to just using liquid cell culture as well as immobilisation on membranes as in the case of O_2 electrode based biosensors. It has been reported that the beads can be reused by storage in PBS at 4 °C for as long as 85 days without much loss in activity of the entrapped cells.

Glucose-glutamate mixture is commonly used as standard for calibration of BOD5 values. So, the cells immobilised in sodium alginate beads were subjected to varying concentrations of BOD standard (GGA) solutions in 96 well microplate. As expected, with the increase in the concentration of GGA solutions, the bioluminescence was found to be increasing. The image was captured using Chemi Doc (Biorad) shows the marked gradation in luminescesnce (Fig.5).

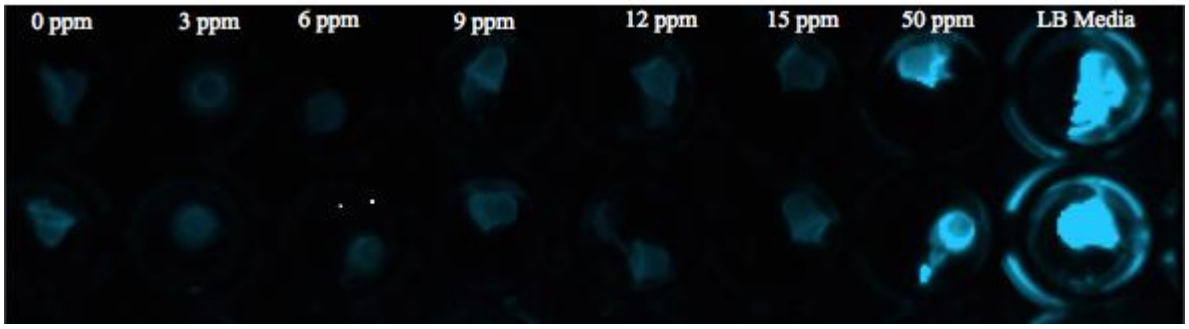

Fig. 5. Gradation in bioluminescence with variation in concentration of GGA. Here, 0 ppm GGA and LB media are taken as positive and negative controls respectively.

4. CONCLUSION & FUTURE WORK

This study attempts to develop a novel microbial biosensor for monitoring the level of biodegradable organic pollutants. Here, we optimized the induction conditions as well as the best temperature for the biosensor.
We next aim to capture the bioluminescence by ordinary camera and make a hand held model which can be operated using Android device.

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