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Application of green fluorescent protein signal for effective monitoring of fermentation processes

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ABSTRACT

This research has focused on the application of green fluorescent protein (GFP) as a new technique for direct monitoring of fermentation processes involving cultured bacteria. To use GFP as a sensor for pH and oxygen, percentage ratio of red fluorescence to green (%R/G) was evaluated. Assessing the magnitude of the %R/G ratio in relation to low or high pH and oxygen concentration, the bacterial strains were cultivated under aerobic and anaerobic conditions. SCC1 strains of E. coli were grown in a 5 L laboratory fermenter, and during the fermentation, the pH and temperature were controlled at 7.0 and 37^{0}C respectively. Dissolved oxygen tension (DOT) was controlled between 15-100% by changing the agitation speed between 20-500rpm respectively. Effect of reducing the DOT level from 100% to 15% was observed after 4.5h fermentation. There was a growth arrest as indicated by the decrease in the OD_{650} at this time (4.5-5h). The relative fluorescence (green) intensity was decreased from about 460 to 420 RFU. But %R/G ratio was significantly increased from about 0.1% to about 0.25% when the DOT level was decreased to 15%. But when the DOT was changed to 100%, a little increase in the RF and decrease in the %R/G ratio were observed. Therefore, GFP can effectively detect and indicate any change in pH and oxygen level during fermentation processes.

Keywords: *Escherichia coli* SCC1, Fermentation process, Green Fluorescent Protein, Red fluorescence

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INTRODUCTION

In the last decade, novel molecular biology techniques based on green-fluorescent protein (GFP) that originated from jellyfish; *Aequoreavictoria* [1] have been observed [2]. Since from the first cloning of the *gfp-gene*, the *A. victoria* GFP and its variants became powerful tools in cell biology and biosystem engineering.

This fluorescent technique employing GFP and its variants has distinctive characteristics of real time detection, safe to the host cells and does not require any cofactor, hence, make GFP applicable for quantitative and qualitative detection methods [3]. Randers-Eichhorn*etal* [4] developed first GFP sensor for quantitative monitoring of GFP fusion protein production from *Escherichia coli*. The outcome has shown that the fluorescent intensity could be used to quantify the *E. coli* biomass. GFP detection has been successfully employed in the field of bioengineering, such as yeast and *E. coli* high cell density cultivation monitoring [5].

Many sensors that can provide information on O_2 concentration, pH values and other essential features of both intracellular and extracellular environment have been developed from proteins that are GFP variants [2].

MATERIALS AND METHODS

A.Bacterial Strains and Plasmid
The strain of *E. coli* used in this research was SCC1 (contains *gfp-gene* in its chromosome) obtained from Biochemical Engineering Unit, School of Chemical Engineering, University of Birmingham, UK.

B. The Media
Lennox-broth (LB) was used which contains $5gL^{-1}$ yeast-extract, $10gL^{-1}$tryptone, $5gL^{-1}$NaCl and distilled water. The prepared media was sterilized before use in an autoclave (Series-300, LTE-Scientific Ltd; Greenfield) at 121^0C for 15minutes.

C. Glucose Stock Solution
40% (w/v) glucose stock solution was prepared and sterilized separately at 121^0C for 5minutes.

D. Batch Fermentation
The *E. coli* SCC1 was grown aerobically overnight in an incubator-shaker (Innova 4000, NBS, USA) at 37 ^{0}C and 200 rpm. The growth was in 250 mL conical flasks (duplicate) containing 30 mL working volume of LB medium. 30 mL of this overnight (seed) culture was inoculated into 5 L laboratory scale fermenter (Fermac 310, Electrolab Ltd, England) containing 3 L working volume of LB medium supplemented with phosphate buffer (3.8 gL^{-1} KH_2PO_4 and 12.5 gL^{-1} K_2HPO_4) (pH 6.94 at 25^0C), 0.4% (w/v) glucose and 8 drops of antifoam (polypropylene glycol). In the fermentation, aeration, agitation, dissolved oxygen (DO), pH and temperature were controlled online at 0.1-1 vvm, 20-500 rpm, 15-100%, 7.0 and 37 ^{0}C respectively. pH control was achieved through automated addition of 10% (v/v) NH_3 and 5% (v/v) HCl.

Culture-samples were collected at different intervals for 8 hours and finally at 24th hour and analyzed for optical-density at 650 nm (OD_{650}), green fluorescence and red fluorescence.

E.Analytical Methods
OD_{650} measurements for biomass evaluation were carried out by using spectrophotometer (Uvikon-922, Kontron-Instruments; Buckinghamshire, England) and growth rate was calculated.

The culture fluorescence measurements were performed with fluorescence spectrophotometer (LS50B model, Perkin-Elmer Ltd, UK) with Slit-Width at 7.5 nm (excitation) and 15.0 nm (emission), and Integrate Time (IT) at 5s. Green and red fluorescence were measured respectively at 480 nm/510 nm (excitation/emission) and 525 nm/600 nm (excitation/emission). Relative fluorescence (RF) (absolute fluorescence/OD_{650}) and percentage of red fluorescent to green (R/G (%)) were calculated.

RESULTS AND DISCUSSION

A. Bacterial Strain
Fig.1 shows the bacterial cell OD_{650} profile. They were grown under aerobic and anaerobic conditions.

B.The Percentage Red Fluorescence to Green (%R/G)
To investigate the GFP application in direct monitoring of oxygen concentration pH and agitation, the GFP expressing *E. coli* cells under aerobic and anaerobic conditions were strongly photoactivated with blue light. After photoactivation, the magnitudes of the percentage red fluorescence to green (%R/G) ((red fluorescence / green fluorescence) X 100) were evaluated.

During the fermentation, the pH and temperature were controlled so as to minimize/eliminate the low pH effect on the GFP red and green fluorescence intensities. As shown in Fig. 2, pH and temperature were controlled at 7.0 and 37^0C respectively.

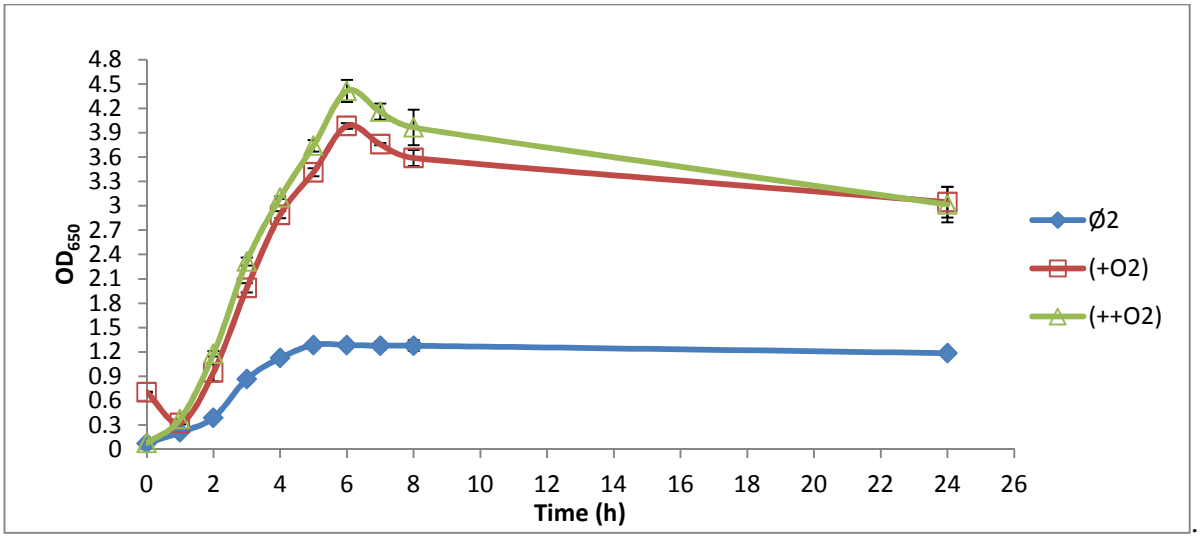

Fig. 1: Comparison of growth rates aerobically and anaerobically. The maximum specific growth rates are 0.5 h^{-1} [++O_2], 0.5 h^{-1} [+O_2] and 0.34 h^{-1} [Ø$_2$]

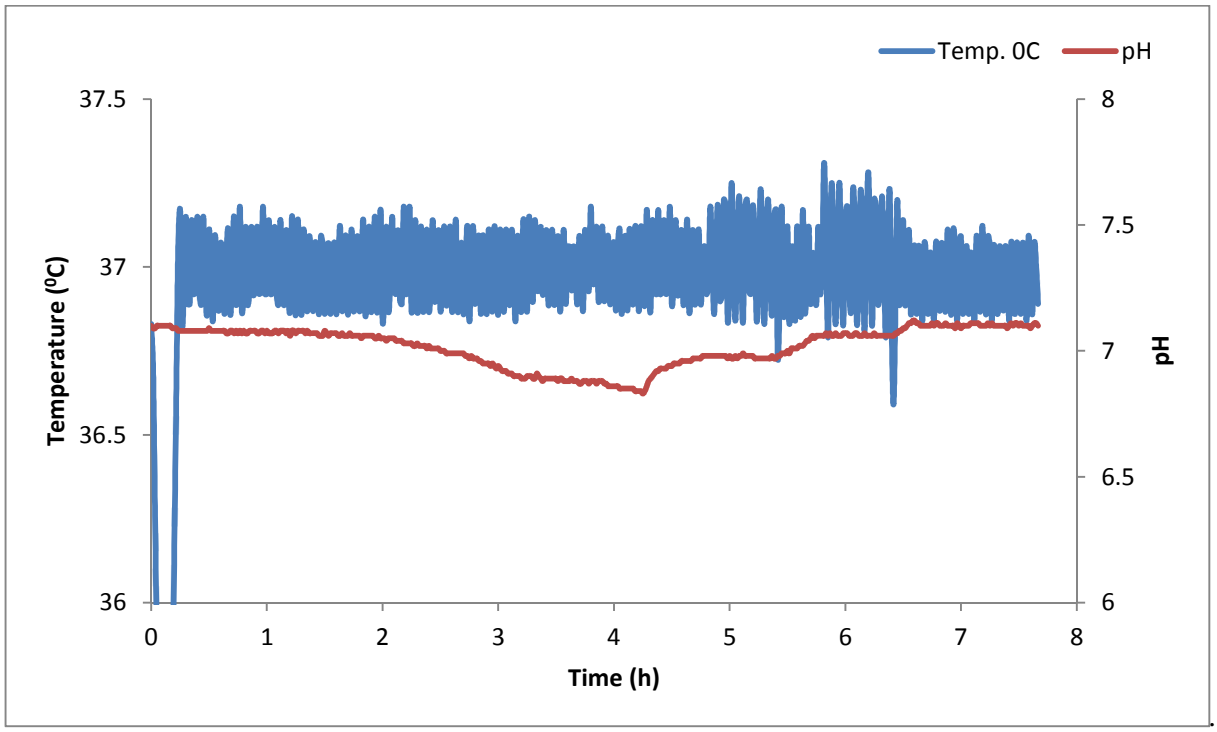

Fig. 2: pH and temperature control

During the fermentation, dissolved oxygen tension (DOT) was controlled between 15-100% by changing the agitation speed between 20-500 rpm respectively as shown in Fig. 3.

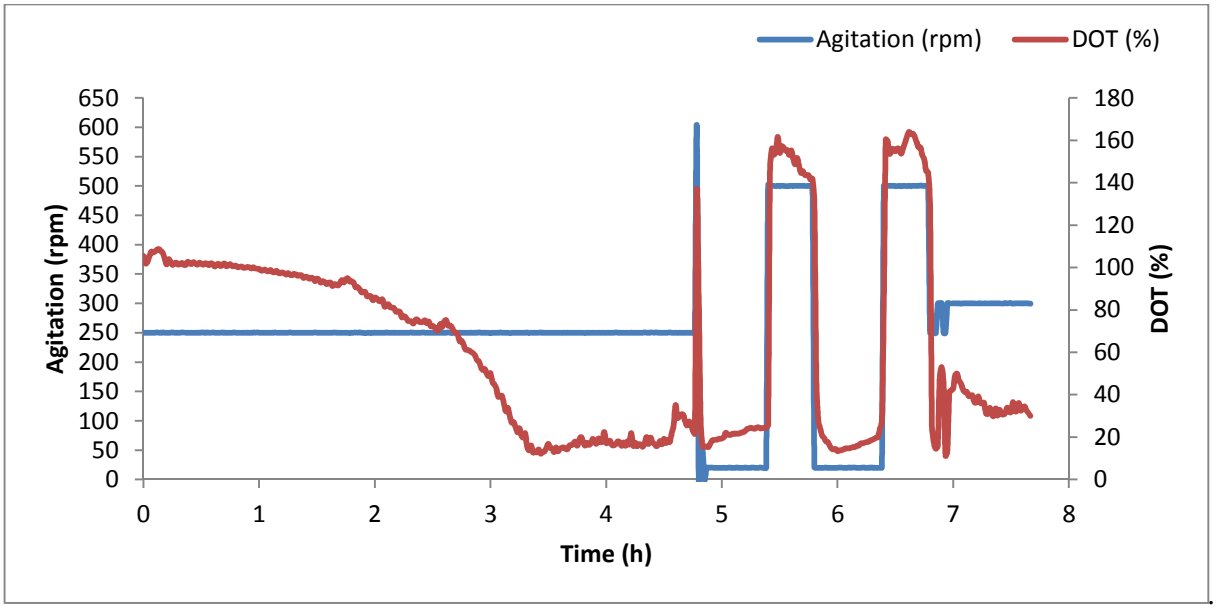

Fig. 3: Agitation and DOT (Dissolved Oxygen Tension) control

Effect of reducing the DOT level from 100% to 15% was observed in Fig. 4 and Fig. 5 after 4.5h fermentation. There was a growth arrest as indicated by the decrease in the OD_{650} at this time (4.5-5h) (Fig. 4). The relative fluorescence (green) intensity was decreased from about 460 to 420 RFU. But %R/G ratio was significantly increased from about 0.1% to about 0.25% when the DOT level was decreased to 15% (Fig. 5). But when the DOT was changed to 100%, a little increase in the RF and decrease in the %R/G ratio were seen.

Quantification and monitoring of oxygen and pH levels are of high importance in bioprocess engineering especially for process optimization and control.

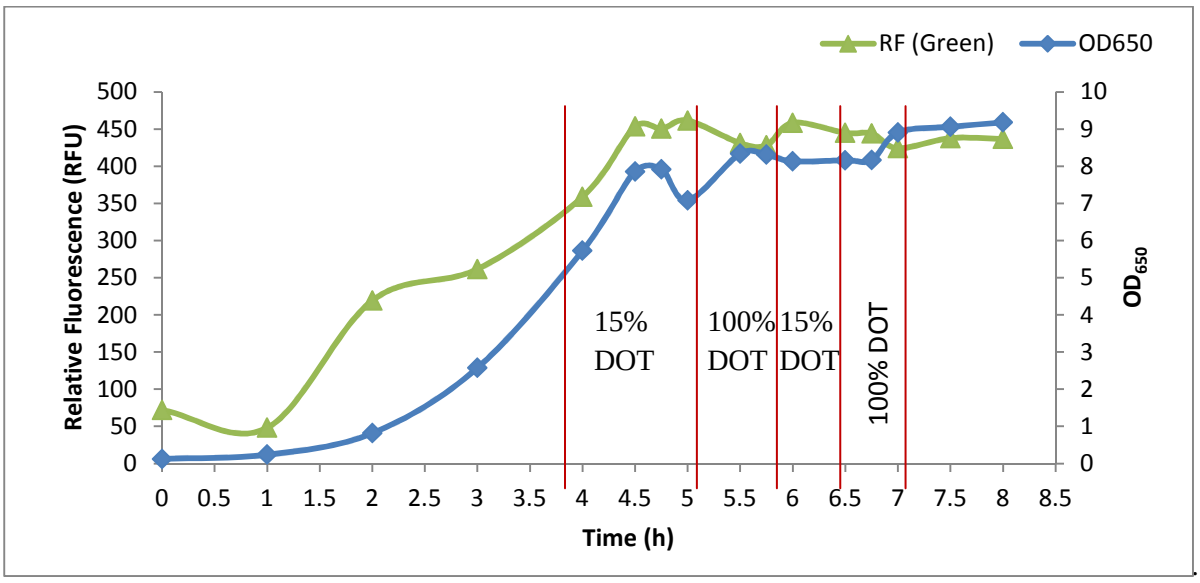

Fig. 4: OD_{650} and Relative-fluorescence (green) intensity. The maximum specific growth rate = 0.91 h^{-1}

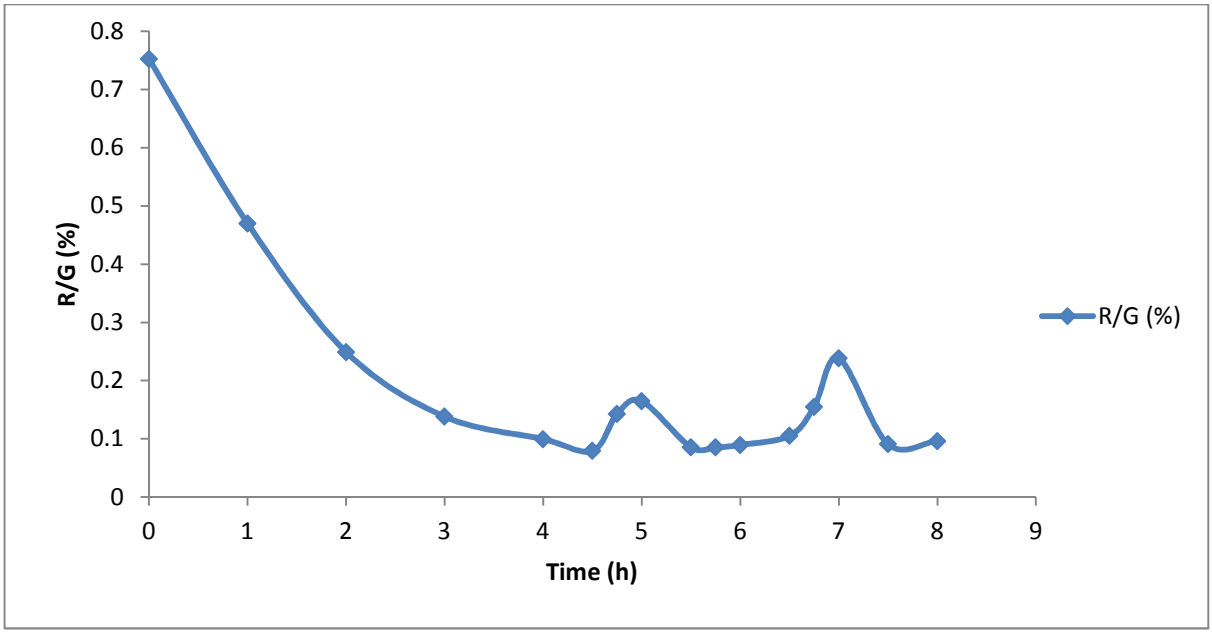

Fig. 5: Percentage red-fluorescence to green (%R/G) profile

Quantification and monitoring of oxygen and pH levels are of high importance in fermentation processes especially for optimisation and control.

In recent time, GFP and its variants have been employed successfully for evaluating oxygen heterogeneities within tissues and within cells under both anaerobic and aerobic conditions [6]-[7].

As seen during the fermentation, increase in the magnitude of %R/G to about 0.25% (Fig. 5) was only occurred when the dissolved oxygen tension (DOT) was reduced to 15% (Fig. 4). But when the DOT was kept between 30-100%, the level of the %R/G was decreased to less than 0.1%.

CONCLUSION

Although its mechanism at the molecular level in anaerobic conditions is not properly understood presently, GFP fluorescence red shift can be used for effective monitoring and control of pH and oxygen concentration. Employing this GFP fluorescence red shift through evaluating the magnitude of the percentage red fluorescence to green (%R/G) in relation to oxygen and pH sensing has been demonstrated. The validity and feasibility of using this method in the direct oxygen and pH sensing in GFP expressing *E. coli* cells was evaluated. With this rapid and real time technique, oxygen concentration and pH levels can be determined especially in bioprocess optimization and control. As long as dissolved oxygen tension (DOT) is kept above 30% in fermentation processes involving GFP expressing cells, the %R/G ratio remains significantly at minimal level (<0.1%). But any decrease in the DOT from ≤15% will be indicated by the rise in the %R/G values above 0.1% as seen in the batch fermentation of the *E. coli* SCC1 strain.

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