Bioreporters as Novel Analytical Sensing Tools

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Abstract- **A bioreporter is a living microorganism containing a sensor molecule that upon binding of a small molecule of interest switches on a reporter, resulting in a measured cellular signal outputs which can be a colorimetric, bioluminescent, or fluorescent emission. They are very specific for the target chemical molecule. The use of bioreporters in detecting target molecules lies in altering the transcriptional regulator so as to change the specificity. Bioreporters are applied in water quality control and assessment, identification of pathogenic organisms of human health concern, to establish toxicity profiles in environmental samples, specific detection of pollutant and heavy metals, determine bioremediation rates, search for novel biocatalysts, and to improve strains for industrial production of small molecules. They are easy to use, rapid, adaptive, and robust tool for chemical analysis. The review highlights the type of bioreporters currently in use, mechanism of switch on and off, and their applications.**

Keywords**: Analysis, Bioreporters, chemical, Sensors, emission**

I. INTRODUCTION

A bioreporter is a microorganism that is activated by an external molecule to produce a detectable cellular signal [1]. The signal outputs can in the form of a color, bioluminescence, or a fluorescence light which indicate target chemical presence or a biological process that could be measured. The bioreporter has high specificity for a target molecule which triggers a measurable outcome thus making the system amenable to manipulation hence its use for high throughput screening[2], [3].Microorganisms as bioreporters are mostly genetically engineered to produce dose-dependent quantifiable signal in response to the presence of a specific or groups of substances or stress factors in the environment. Most bioreporters for environmental monitoring targets contaminants as relating to water quality and toxicity profiles, identification of heavy metals and organisms of human health concern[4].

The use of bioreporters is on the increase due to its high specificity, high enantioselectivity, reduced cost and handling, online measurement and signal enhancement, coupled with no requirement of artificial substrate it embraces [5],[6]. Signals produced by microbial bioreporters have been adopted to monitor cell populations and responses to other stimuli in the environment. The bacterial luxCDABE operon as an example is operational in many bacterial species with the ability to produce bioluminescence light. It works by producing enzyme luciferase and the substrate required for production light energy without depending on an external substrate sources. Replacement of the luxCDABE promoter gene with another gene of interest can be used to monitor changes in gene expression as a function of bioluminescence and bacterial survival[7],[8].

Bioreporters have two parts, a sensor which function to transcribe and translate messages from the DNA to the mRNA, or determine the type of protein to form and the reporter part which expresses a phenotypic characteristics into a detectable signal [6](van Rossum*et al*., 2017). Specificity of a bioreporter is essential for its normal functioning though obtaining it is laden with challenges such as poor or no expression when many analytes are involved, loss of protein stability, and poor translation to field, and at the different levels, it could be time consuming [6], [9], [10],[11]. In spite of these, its use in different associated field of science is increasing and enormous effort is being made to surmount the challenges highlighted above[3][12].

II. TYPE OF BIOREPORTERS

Bioreporters are mostly described according to their output as colorimetric, fluorescent and bioluminescent.

a) Colorimetric Bioreporters

The *lacZ* gene or β-Galactosidase is obtained genetically from *Escherichiacoli* and it encodes a β-galactosidase (*β-gal*) enzyme that mediates the hydrolysis of substrate β-galactoside disaccharides (lactose) into monosaccharides (glucose and galactose). Onitrophenyl-β-D-galactoside (ONPG) causes *lacZ* to produce a colorimetric output which makes it a veritable bioreporter. *lacZ*gene can fuse to a chemical-responsive promoter which changes color when chromophores is introduced to an assay medium. Thus the color density can be measured on a standard spectrophotometer which makes the bioreporter inexpensive and useful for qualitative or quantitative assays. Kits are presently available for monitoring toxic compounds in environmental samples; and the bioreporters can also be manipulated to produce luminescent, chemiluminescent, or fluorescent outputs[13].

b) Fluorescent Bioreporters

Fluorescent bioreporters are engineered using green fluorescent protein (GFP) produced by *Aequoreavictoria*. GFP is a natural and recombinant photo-proteins activated by an external light source to produce a palette of colors[14]. At different excitation wavelength, different versions of GFP (blue-, red-, and yellow-shifted variants) fluoresce. It is used as a bioreporter in eukaryotic systems for its simplicity and quantification is by the use of a fluorescent spectrophotometer or plate reader. It has the advantage of using multiple bioreporters simultaneously. Bioreporters with GFP adoption can be applied to evaluate the environment, and the separate colored light emission signals can be indicative of different outcomes[15].

c) Biolumiscence Bioreporters

Bioluminescence is the generation of light within a living organism through series of chemical reaction catalyzed by luciferase enzyme on luciferin (a substrate) to produce an excited molecule that generate photons. The two common luciferase/luciferin reactions adopted as bioreporters are the bacterial bioluminescent system (lux) and the firefly bioluminescent system (luc).

i) Bacterial luciferase (lux): In bacterial luciferase system, molecular oxygen oxidizes riboflavin phosphate (FMNH2) in association with a long chain aliphatic aldehyde in a reaction catalyzed by luciferase enzyme to an aliphatic carboxylic acid. The reaction forms an excited hydroxyflavin intermediate, which is dehydrated to the product FMN which emits blue-green 490 nm light signal. The reaction is controlled by a five gene operon consisting of the luxA, luxB, luxC, luxD, and luxE genes. WhileluxA and luxB (luxAB) gene products form heterodimeric luciferase, luxC, luxD, and luxE (luxCDE) gene products supply and regenerate the long-chain aldehyde needed for the reaction. The required molecular oxygen and FMNH2 reactants are sourced within the cell through supporting metabolic processes.

Two classes of lux-based bioreporters used are; i) the one that integrates only the luxAB genes, with the luciferase enzyme and requiring an external aldehyde source. The light signal output is brighter and easier to detect due to substrate saturation. The design is common in bacterial, yeast, and mammalian genetic systems and remain well tested within environmental, food, and water-based bioassays; ii) theluxCDABE gene produces bioluminescent signals using independent substrate supply without external intervention which gave the bioreporter such attribute of real-time to near real-time detection capabilities. The luxCDABE genetic operon has been genetically optimized for efficient gene expression thereby allowing for its integration into a wider variety of bacterial hosts [16], and gene regulation in mammalian cells [17].

ii) Firefly luciferase (luc): The luc gene is commonly found in firefly *Photinuspyralis* and click beetles with the capacity to produce high light output. The enzyme catalyses the oxidation of luciferin, requiring in the presence of oxygen and ATP. Oxygen molecule combines with calcium, adenosine triphosphate (ATP) and a substrate (luciferin) in the presence of light-emitting luciferase enzyme to produce a bioluminescent light.

The chemical reaction catalyzed by firefly luciferase takes place in two steps:

luciferin + ATP → luciferyladenylate + PP_i

luciferyladenylate + O_2 → oxyluciferin + AMP + light

Light is produced because the reaction forms oxyluciferin in an electronically excited state. The reaction releases a photon of light as oxyluciferin goes back to the ground state. Luciferyladenylate can additionally participate in a side reaction with oxygen to form hydrogen peroxide and dehydroluciferyl-AMP. Firefly luciferase generates light from luciferin in a multistep process. First, D-luciferinis adenylated by MgATP to form luciferyladenylate and pyrophosphate. After activation by ATP, luciferyladenylate is oxidized by molecular oxygen to form a dioxetanone ring. A decarboxylation reaction forms an excited state of oxyluciferin, which tautomerizes between the keto-enolforms. The reaction finally emits light as oxyluciferin returns to the ground state. luc reporter systems have the disadvantage of requiring addition of exogenous luciferin substrate, which hinders automation in a continuous fashion.

III. PRINCIPLE OF OPERATION A BIOREPORTER

A bioreporter is made up of a reporter gene and a regulatory protein. It exerts its action based on the fusion of a specific promoter gene with a reporter gene which initiates transcription of mRNA and production of protein that generate detectable signal. The reporter gene controls transcription and production of protein which are able to detect an analyte. A reporter gene as sensors can transform a biological response into a detectable signal which is important for the sensitivity and selectivity of a bioreporter. Presently adopted reporter genes include *lux*I, *lac*Z, *gfp*, *dmp*R. The regulatory proteins on the other hand interacts with target analytes to obtain e that is measurable. Regulatory protein aid specificity and sensitivity of the bioreporter.

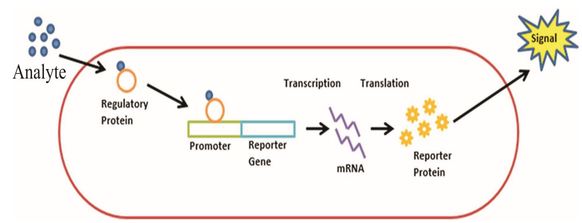

Figure 1: Schematic diagram of the mechanism of operation of abioreporter

Bioreporters work on either a light-off or light on system. In the light-off system, the promoter gene which ordinarily regulates expression of bolumiscent, fluorescent, or colorimetric light on exposure to an unfriendly analyte produces reduced signal or light –off response corresponding to the concentration of such a toxic analyte in the environment.

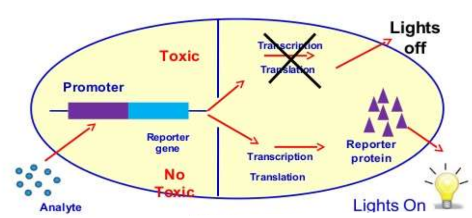

Figure 2:Schematic diagram of the lights-off mechanism in a bioreporter

In a light on bioreporter system, the signal is activated when an analyte or a targeted chemical come in contact with the microorganism. The presence of a target analyte causes fusion between an inducible promoter and a promoter gene that initiates transcription/translation which results in the reporter protein producing a detectable signal.

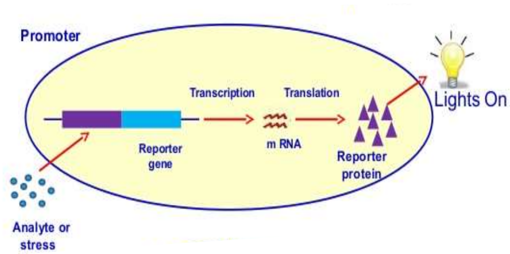

Figure 3: Schematic diagram of the lights-on mechanism in a bioreporter

IV. APPLICATIONS OF BIOREPORTERS

Bioreporters have been reported to be of innumerable uses in industries, environmental monitoring, and in research studies. According to Leonard*et al*. [18], Diplock*et al*. [19] and others, applications of bioreporters can generally be classified into four major groups below;

1 Detection and identification of substances in the environment

A host of substances released into the environment by industries and production facilities have toxic and unfriendly health effects on man and the environment

a) Evaluation of toxicity levels in the environment

Bacterial cells can be manipulated to detect the presence or availability of xenobiotic chemicals and the imposed toxicity in the environment [20],[21. Bioreporters can be designed to determine and evaluate mutagenic, genotoxic, and cytotoxic effects of a chemical compound, and also determine the oxidative stress such compound can impose on cells. Genotoxins induce production of reactive oxygen species which causes DNA damage and mutagenesis. Bioreporters have been developed with capacity to sense, encode detectable proteins, and while characterizing chemicals and quantifying their concentration in the environment.Whole-cell bioreporters have been designed to detect genotoxic chemicals and evaluate individual effects or synergistic impacts of couples of chemicals at affordable cost [22],[23].

b) Assessment of heavy metals in soil and water environment

Water is a scarce commodity in several place in the world, and it is becoming more challenging due to industrial and agricultural release of chemicals and other toxic substances into water bodies thus endangering aquatic and human life. Arsenic and lead contamination of water has been variously reported in the environment [24],[25]. Bacterial bioreporters have been designed that can assess and evaluate the contamination level of these heavy metals and others in water and other aquatic environment. In the soil, it is adopted for monitoring heavy metals such as nickel, lead and other chemical contaminants[26], [27], [28], [29], [30], [31],[32].

c) Evaluating pollution on land and in aquatic environment

Bioreporters are applied in the detection of pollutants in the environment [33]. Bacteria strains have also been designed to help monitor xenobiotic substances such as *Burkholderiasartisoli* RP007 (pPROBE-*phn-luxAB*), *E. coli* DH5α (pHYBP103M3), and *E. coli*pGLTUR with the capacity to monitor naphthalene and phenanthrene, 2-hydroxylbiphenyl and biphenyl, and toluene respectively in soil[34], [35], [36].

2 Industrial applications of bioreporters

Bioreporters have been variously designed for use in the industries to aid production of goods for further use by man and in research endeavors.

a) Identification of promising biocatalyst[37].
b) Production and detection of small molecules[6], [38].

3 Study of microorganisms in relation to human pathogens and disease conditions

a) Some bacteriophage are also adopted bioreporters applied in the identification of pathogenic organisms of human health concern [25],[39].
b) Monitoring biofilm production and pathogenic bacteria [40].
c) Monitoring cancerous growth in man and animal models[41], [42], [43], [44].

4 Determining roles of microorganisms in plant soil interactions

a) Monitoring of several plants pathogens [45].
b) Monitoring of physiological status of cells with respect environmental stress factors[46].

5 Applications in bioremediation

Bioreporters are reportedly used in bioremediation and biodegradation monitoring[47], [48],[49].

V. CONCLUSION

Bioreporters are novel complementary analytical and evaluating machineries with a robust information processing and online monitoring capabilities. The use of these tools have proven popular especially in environmental monitoring where large expanses of land and water bodies are evaluated for different parameters. The advantages it has over other analytical techniques make them particularly endearing to use in its easy to use and adaptability to varied experimental situation.

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