Bioreporters as Novel Analytical Sensing Tools

Tony Oyigbeche¹, Olukayode Olugbenga Orole¹, Victor Stephen Fadayomi²

¹Department of Microbiology, Federal University of Lafia, Nigeria
²Department of Zoology, Federal University of Lafia, Nigeria

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 Escherichia coli and it encodes a β-galactosidase (β-gal) enzyme that mediates the hydrolysis of substrate β-galactoside disaccharides (lactose) into monosaccharides (glucose and galactose). ONitrophenyI-β-D-galactoside (ONPG) causes lacZ to produce a colorimetric output which makes it a veritable bioreporter. lacZgene 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 Aequorea victoria. 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
The chemical reaction catalyzed by firefly luciferase takes
bioluminescent light.

The 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
(FMN2) 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. While luxA 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)
the luxCDABE 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:

\[
\text{luciferin + ATP \rightarrow luciferyladenylate + PP}_i
\]

\[
\text{luciferyladenylate + O}_2 \rightarrow \text{oxyluciferin} + \text{AMP} + \text{light}
\]

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 luciferyl adenylate
and pyrophosphate. After activation by ATP, luciferyl adenylate is oxidized by molecular oxygen to form a
dioxetanone ring. A decarboxylation reaction forms an excited
state of oxyluciferin, which tautomerizes between the keto-
ens. 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, lacZ, gfp, dmpR. 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.

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.
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], Diplocket 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-E-plm-luxAB), *E. coli* DH5α (pHYBP103M3), and *E. coli*pGLTUR with the capacity to monitor naphthalene and phenanthrene, 2-hydroxybiphenyl 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.
REFERENCES


