

**UNITED STATES PROVISIONAL PATENT
APPLICATION FOR**

Bioreactor to Modulate between Nitrogen and Oxygen
Environments for PAH Degradation

Inventor(s): Christopher Caligiuri, Emily Kang, Andrew Gao

MAILING ADDRESS:

CCA iGEM

5951 Village Center Loop Rd

San Diego CA 92130 US

Phone: 8583569456

Applicant's Ref. No: 657

FIELD OF THE INVENTION

The present invention relates to the field of mechanical, software, and process design. Specifically, the present invention relates to a novel methodology and apparatus for modulating between two different environments required for PAH degradation.

BACKGROUND OF THE INVENTION

In 1969, an oil well located off the coast of Santa Barbara experienced a blowout, leading to one of the largest oil spills ever charted in U.S. waters. This disaster, which made headlines nationwide, had profound effects on marine life, with oil from underwater fissures creating a slick that would eventually cover an area the size of Chicago. It only took a few days for the oil to reach the beaches of Santa Barbara, killing thousands of birds and contaminating the waters; ultimately, this resulted in drastic changes in legislation.

A synthetic system utilizes the natural ability of microorganisms to degrade some of the most prevalent, toxic PAHs, a predominant component of crude oil, into harmless compounds. The system then reuses these catabolic end products by taking advantage of bacterial metabolism to produce clean energy by coupling the degradation pathways with sequences that upregulate hydrogen synthesis within *E. coli*. In addition, we use synthetic pathways to metabolize long n-chained hydrocarbons to fuel such hydrogen synthesis. To modulate between the different environments needed for PAH degradation and hydrogen synthesis, a bioreactor was designed. Therefore, what is needed are techniques that overcome the above mentioned disadvantages.

SUMMARY OF THE DESCRIPTION

The invention utilizes temperature and pressure sensors in order to effectively modulate between the two environments. The first environment requires nitrogen, which promotes anaerobiosis; after bubbling, it maintains a pressurized environment via the use of a pump. The second environment requires oxygen, which also maintains pressure via a

pump. The Arduino contains custom designed software that monitors the pressure of the bioreactor and pumps in gas as necessary. It also utilizes a temperature sensor that maintains optimal thermal conditions within the bioreactor. Finally, a dissolved oxygen sensor is used to monitor the oxygen concentration within the bioreactor for aerobic reactions. The vessel itself has two stainless steel caps with a plexiglass vessel. There is another separate component that is attached inside the main vessel: the stirring mechanism. It is also displayed in this document. If money is a significant constraint, the bioreactor could also be 3D printed.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is illustrated by way of example and not limitation in the figures of the accompanying drawings in which like references indicate similar elements.

Figure 1 illustrates a sideways, angled view, according to one embodiment of the present invention.

Figure 2 illustrates a bottom, angled view, according to one embodiment of the present invention.

Figure 3 illustrates a side-bottom focused view of the device, according to one embodiment of the present invention.

Figure 4 illustrates a top angled view of another separate component of the device, according to one embodiment of the present invention.

Figure 4 illustrates a bottom angled view of another separate component of the device, according to one embodiment of the present invention.

DETAILED DESCRIPTION

Various embodiments and aspects of the inventions will be described with reference to details discussed below, and the accompanying drawings will illustrate the various embodiments. The following description and drawings are illustrative of the invention and are not to be construed as limiting the invention. Numerous specific details are described to provide a thorough understanding of various embodiments of the present invention. However, in certain instances, well-known or conventional details are not described in order to provide a concise discussion of embodiments of the present inventions.

Reference in the specification to “one embodiment” or “an embodiment” or “another embodiment” means that a particular feature, structure, or characteristic described in conjunction with the embodiment can be included in at least one embodiment of the invention. The appearances of the phrase “in one embodiment” in various places in the specification do not necessarily all refer to the same embodiment.

The present invention will now be described in detail with reference to embodiments thereof as illustrated in the accompanying drawings.

The invention utilizes sensors controlled by an Arduino board to create optimal environmental conditions required for the outlined reactions to occur. It maintains optimal temperatures, pressure, and relative concentrations of nitrogen and oxygen. The Arduino board, which acts as the microprocessor, is able to control a stepper motor which controls a stirrer, as well as the two pumps which control the concentrations of nitrogen and oxygen. This allows for the two different reactions to occur: the degradation reactions and hydrogen synthesis reactions.

This invention could be used to modulate between two different required environmental conditions, one that requires nitrogen and one that requires oxygen. It could be utilized in a variety of methods, as reactions often require different temperatures and other intermediates.

The below figures outline the design of the bioreactor, with all the functional elements outlined through them.

It should be apparent from this description that aspects of the present invention may be embodied, at least in part, in software. The techniques may be carried out in a computer system or other computer system in response to its processor, such as a microprocessor, executing sequences of instructions contained in memory, such as a ROM, DRAM, mass storage, or a remote storage device. In various embodiments, hardware circuitry may be used in combination with software instructions to implement the present invention. Thus, the techniques are not limited to any specific combination of hardware circuitry and software nor to any particular source for the instructions executed by the computer system. In addition, throughout this description, various functions and operations are described as being performed by or caused by software code to simplify



Figure 1 illustrates diagram of an embodiment of the device as a whole, as well as the holes strategically placed on the top of the device to allow for gas to escape and the two holes for oxygen and nitrogen input.

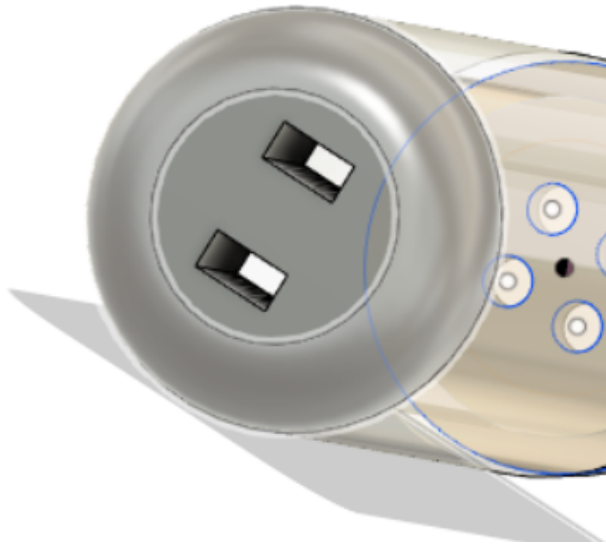


Figure 2 illustrates in greater detail the two holes where nitrogen and oxygen pumps will be inserted to regulate conditions inside the bioreactor.

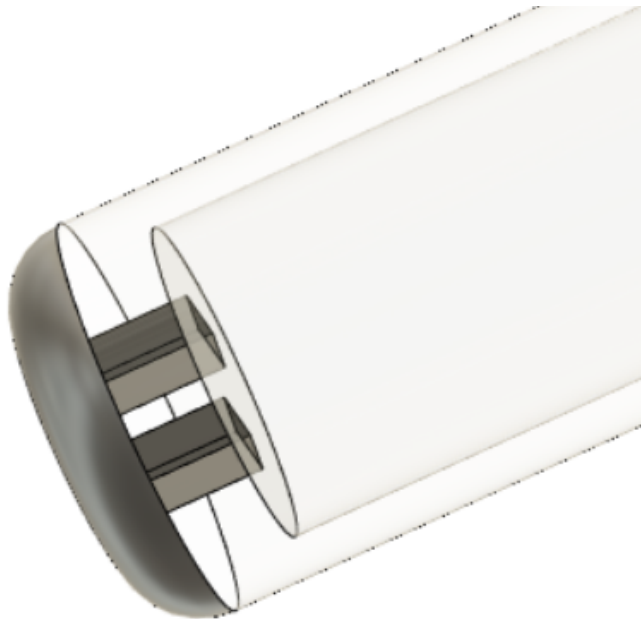


Figure 3 shows the inner chamber of the bioreactor where the reactions will take place. The previously mentioned nitrogen and oxygen pump holes feed directly into this chamber.

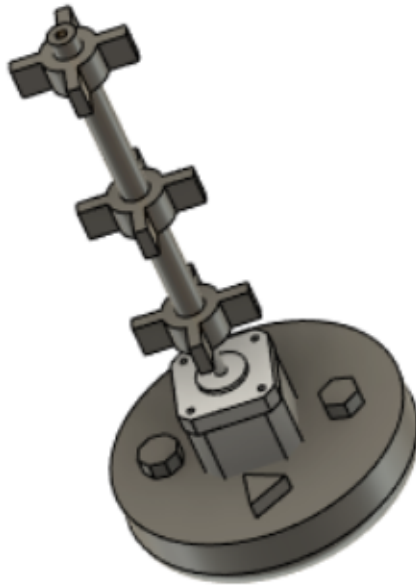


Figure 4 illustrates the unique stirring mechanism of the bioreactor which is inserted into the inner chamber. The central box shape is a stepper motor (Openbuilds 2019) which spins the long rod, which has blades attached. The hexagon, octagon, and triangle, are the pressure sensor, temperature sensor, and dissolved oxygen sensor, respectively.

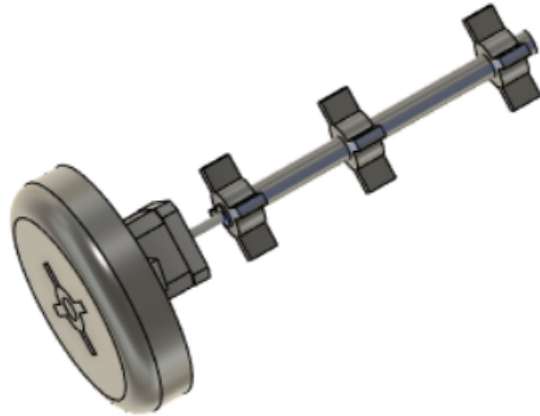


Figure 5 illustrates the stirring mechanism of the bioreactor which is inserted into the inner chamber. The bottom of the mechanism houses an Arduino and sensors, and it is curved to fit into the curved base of the bioreactor.

CLAIMS

1. A bioreactor comprising:
 - a stirrer;
 - a temperature sensor;
 - a dissolved oxygen sensor;
 - a nitrogen concentration sensor;
 - a stepper motor;
2. A method comprising:
 - regulating O₂;
 - regulating N₂;
 - stirring the contents;

ABSTRACT

The invention utilizes temperature and pressure sensors in order to effectively modulate between the two environments. The first environment requires nitrogen, which promotes anaerobiosis; after bubbling, it maintains a pressurized environment via the use of a pump. The second environment requires oxygen, which also maintains pressure via a pump. The Arduino contains custom designed software that monitors the pressure of the bioreactor and pumps in gas as necessary. It also utilizes a temperature sensor that maintains optimal thermal conditions within the bioreactor. Finally, a dissolved oxygen sensor is used to monitor the oxygen concentration within the bioreactor for aerobic reactions. The vessel itself has two stainless steel caps with a plexiglass vessel. There is an inner component which is the stirring mechanism. If money is a significant constraint, the bioreactor could also be 3D printed.