Experimental Evolution: Adaptation of Microorganisms to Extreme Environments

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Video of the Interview with the Group

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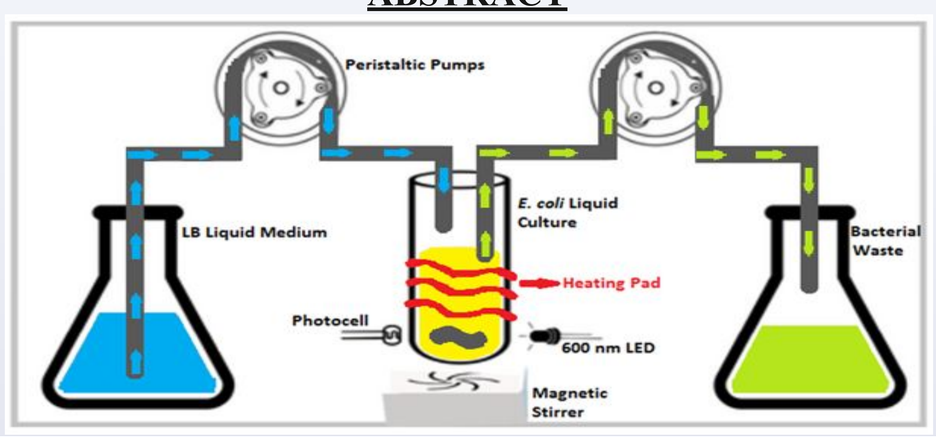
ABSTRACT



Evolution is the change in the genetic composition of populations over time. To observe evolution in a short time period, microorganisms are best candidates because they grow very fast and have large populations. Experimental evolution is a technique used to evolve microorganisms in a laboratory.

Specifically, genetic change or mutations mostly occur due to some random errors during DNA replication of a cell and an organism may gain new abilities after accumulation of them. These abilities may increase the fitness of the organism to the environment. Thus, the main aim while conducting a directed evolution experiment is the observation of microorganisms' adaptation to particular stressful condition.

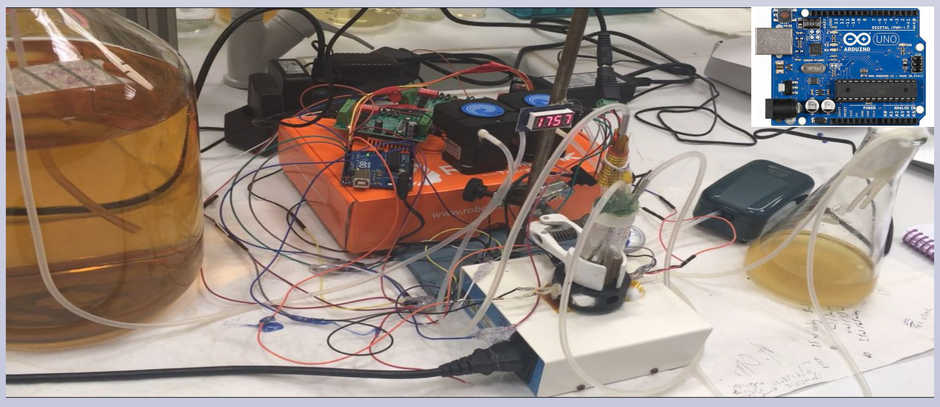
Chemostats and morbidostats are widely used set-ups in directed evolution experiments. Chemostats provide chemically static environment and constant evolutionary pressure, whereas morbidostats provide dynamic conditions that changes the pressure gradually. In this project, parameters of these systems are controlled by Arduino board, which also provides flexibility in designing distinct systems.

OBJECTIVES

Studying experimental evolution with Arduino based morbidostats and chemostats:

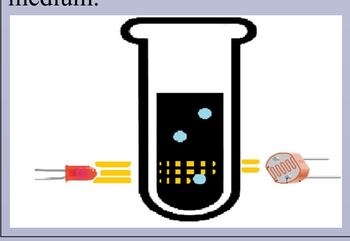
- Research on *E.coli*'s growth rate limitations and its possible adaptations for faster reproduction
- Genetic adaptation of *E. coli* to fix nitrogen from air
- Genetic adaptation of *E.coli* to utilize cellulose as a carbon source
- Genetic adaptation of *E.coli* to catch iron more efficiently in an environment with low-iron concentration

EXPERIMENTAL SET-UP



Arduino board is a hardware which is useful for creating interactive projects. We code in Arduino IDE software and then upload the code into the Arduino hardware. The hardware drives the electronic components of the bioreactor system via electronic circuits and connections.

There are two separate medium containers for fresh medium and waste medium. Two peristaltic pumps creates temporary pressure difference in the hose and moves the medium in the desired direction consequently. One of them adds fresh medium from the fresh medium container into the bioreactor while other one removes the waste medium from the bioreactor into the waste medium container. A temperature sensor and a heating pad are required to keep the temperature of the bioreactor constant, which is 37°C. A magnetic stirrer is very useful for homogeneous spread of the compounds in the medium.



There is a LED at one side of the bioreactor at the bottom of it and there is a photocell at the other side of the bioreactor. Photocell's resistance changes according to amount of light coming onto it. And, we measure the voltage changes depending on the photocell's resistance. It means that, when the bacteria concentration is high enough, light emitted from the LED cannot reach to the photocell effectively and the system increases the evolutionary pressure.

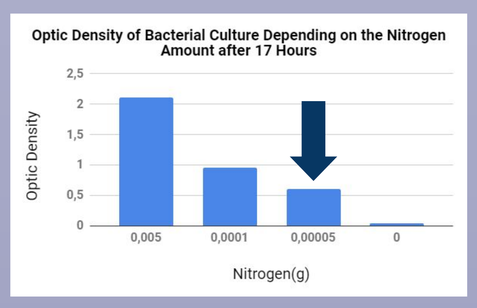
1. INCREASING FLOW RATE

Since some of the bacteria –along with waste medium- are removed from the bioreactor, bacteria must reproduce proportional to the flow rate to sustain their lives in the bioreactor. At the beginning of the experiment, flow rate is arranged such that bacteria reach their physiological reproduction capacity.

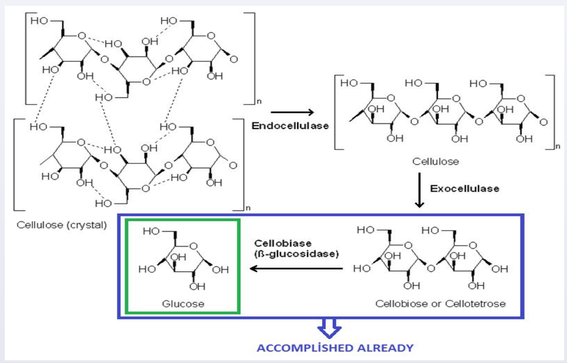
Secondly, the system is started as a morbidostat and the flow rate is increased gradually. So, bacterial population is expected to genetically adapt to the increasing flow rates and reproduce faster under the evolutionary pressure of more bacteria removal.

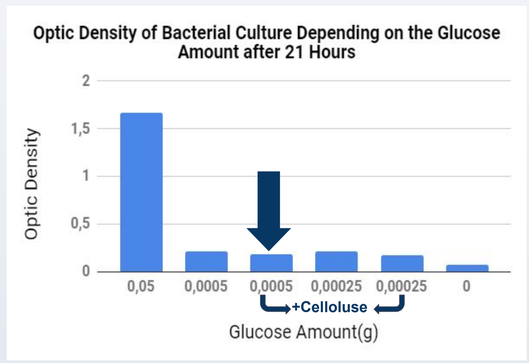
2. NITROGEN FIXATION

Normally, *E. coli* uses the nitrogen sources in its liquid environment however; in a specific case, it has been shown that a strain of it can fix gaseous nitrogen under anaerobic conditions. By the help of directed evolution in a chemostat, we conducted an experiment to do the same thing under aerobic conditions. To create evolutionary pressure, the nitrogen deficient medium was used. The medium with nitrogen concentration where bacteria do not die but also suffer from lacking of nitrogen is selected. OD was followed to monitor the bacterial growth to see whether they gained this ability or not.



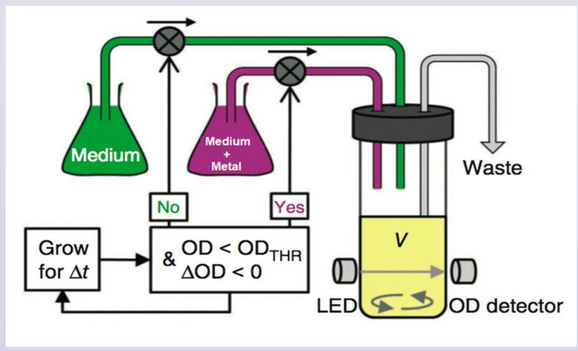
3. UTILIZATION OF CELLULOSE





Glucose is the main source of carbon for *E.coli* under standard conditions. Cellulose, the polymer consisting of glucose, cannot be hydrolysed by *E. coli* due to lack of certain enzymes. On the other hand, cellobiose, a disaccharide similar to cellulose in structure, can be used as the carbon source under evolutionary pressure as shown in previous studies [1]. The purpose of this experiment is to see whether *E. coli* can use cellulose instead of glucose in an environment where the glucose level is restricting the growth rate of bacteria in a chemostat. If bacteria adapt to these conditions, the OD will increase and the culture will be tested for usage of cellulose.

4. INCREASING METAL BINDING CAPABILITY



Iron is one of the essential elements for living organisms. Iron containing proteins participate in transportation, storage and usage of oxygen. However, ferric ions are poorly soluble under aerobic conditions at neutral pH, thus organisms have developed to catch iron as it is in the *E. coli.* In this study, it is aimed to increase the ability of *E.coli* to catch iron by using a morbidostat.

The morbidostat has two different media, which differ only in iron presence. Arduino board sets the concentration of the iron present in the bioreactor by arranging the pump rates of two media. As the *E.coli* adapt to lower level of iron concentration (OD < Threshold OD), arduino will decrease iron concentration for further adaptations.

CONCLUSIONS

- Two chemostats and two morbidostats systems are set up and the experiments are started successfully.
- **Increasing flow rate experiment:** Reproduction rate of bacteria reached to the theoretical reproduction capacity of the strain. However, agglomeration suppressed the evolutionary pressure by preventing the removal of the bacteria that's why the flow rate increased exponentially in day 12-13 [2] (Figure 1-2).
- **Nitrogen fixation experiment:** Continued for eight days however; failed due to contamination in the fresh medium (Figure 3). Therefore, decreasing bacteria concentration is observed in OD values due to lack of sources coming from the nutrient tank (Figure 4).
- **Cellulose utilization experiment:** Before conducting the experiment, preparation of the fresh medium is revised to prevent turbidity in the medium for correct OD measurement. Additionally, because cellulose pieces were splintered due to the mixation in the medium, a piece of cotton was sunk into the medium as a cellulose source to optimise OD measurement. The experiment has been continuing for 11 days, but no change in OD has been measured, which implies that there is not any mutant, able to utilize cellulose, yet (Figure 5).
- **Increasing metal binding capability experiment:** Iron concentration is decreased from 0.2 mm/L to to 0.152 mm/L in 16 hours. At this percentage, iron precipitated in the hose because of lower pump rates. Dilutioning the medium including iron and increasing the pump rate of it the problem was solved without changing the pumped iron amount. However, contamination started at the fresh medium container, thus the experiment is terminated (at right in Figure 6).

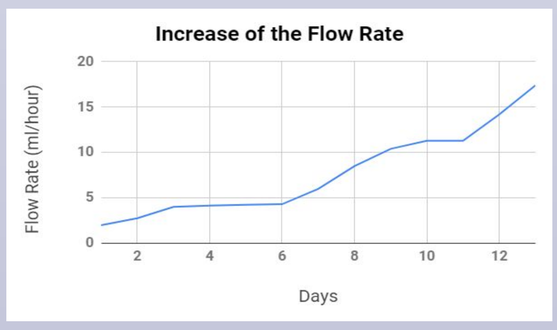


Figure 1

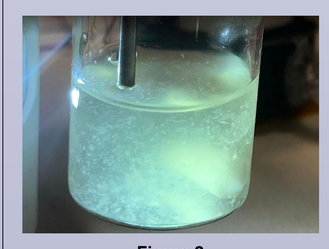


Figure 2

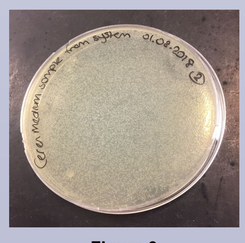


Figure 3

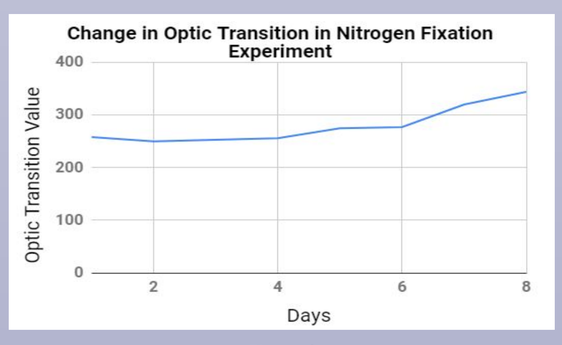


Figure 4

Figure 5

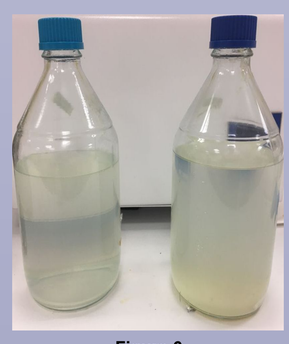


Figure 6

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