**ABSTRACT**

Nowadays, environmental pollution and energy depletion have become crucial problems. We need to find alternative energy to replace the running out fossil fuel. Due to the pollution issues, this alternative energy should be environmentally friendly. To cut down energy, we chose isobutanol to be our project. We believe that isobutanol is a potential eco-fuel in the future.

Our team: NCTU_Formosa, constructed the low temperature release system in E. coli to achieve high yield production of isobutanol as biofuels. Our project, Ecofuel E.coli, provides several optimization methods to improve production rate of isobutanol as follows: (1) A suitable medium was selected. (2) Five E. coli strains were tested. (3) Temperature-controlled RBS was used to precisely control our system. (4) The fusion protein of a target enzyme and a zinc finger protein was constructed to increase the specificity of regulation. Our data reveals that the E. coli strain DH5α grows in M9TY medium for 24 hours at 42°C have highest production of isobutanol (>15 g/L).

We used this pathway to realize our goal, producing isobutanol by low temperature release system.

**CELLULOSE**

In order to realize our idea of changing agricultural wastes into biofuel, we figured that we could try to obtain glucose from cellulose by cellulase. We did a pre-experiment by using quantitative filter paper as cellulose source.

**ZINC FINGER**

In traditional way, the four enzymes dispenses in E.coli, and the reaction proceed by chance, leading to an inefficient isobutanol production system. So, we add zinc finger on our enzymes and link them up on a DNA program. This way, it will accelerate the isobutanol production.

**OPTIMIZATION**

To maximize the isobutanol production, we optimized E.coli strain, culture medium, time, temperature and carbon source. Amazingly, our production surpasses the published references whose production is 5.85g/L for 24 hr by using modified JCC16 strain.

**INTEGRUMENT**

In order to further improve our isobutanol production rate, we developed a simple but useful device to help us collect isobutanol.

We prepare two flasks containing half-filled coolant water and each of them was equipped with a condenser. One fermentation flask and two collecting flasks are linked with pipe. First, we pour air into the circulating flask to strip the isobutanol, and then it flows to the first collecting flask through the pipe. Next, isobutanol will pass through the pipe to the second collecting flask.

**ISOBUTANOL TOLERANCE**

We did an experiment to prove the isobutanol is truly toxic to the E.coli. The data shows that the higher concentration of the isobutanol was in the medium, the lower OD600 value could be obtained. The curve in different color represent different concentration of isobutanol.

**TEMPERATURE CONTROL**

In our isobutanol pathway system (Fig. 1), glucose will be converted into pyruvate through glycolysis. Then, pyruvate will be converted into isobutanol by utilizing four enzymes AlkA, iCvA, iCvD, KivO.

But, the isobutanol and isobutyraldehyde are toxic to E.coli, causing low production rate of isobutanol. Therefore, we designed the low temperature release system that allows the pathway to stop and produces sufficient 2-ketobutyrate before proceed the rest of the pathway. Here we can see that our system really works well (Fig. 2).

As our E.coli is in 37°C, KivO is inhibited by TcR And, by the time E.coli is lowered to < 37°C environment, KivO is expressed and yields the isobutanol. Although E.coli will die in presence of isobutanol, we have already obtained enough our desired intermediate, 2-ketobutyrate. Therefore, more isobutanol could be produced.

In Fig. 3, we discovered that E.coli under 42°C for 24 hours have higher production of isobutanol than under 37°C at the beginning. This result is totally out of our expectation, and the high production really surprised us. In previous studies, numerous synthetic circuits were created to test performance in reliability and consistency, but this process is tedious and time consuming. Our project provides a temperature control method to control the expression of a series of metabolic proteins with one version of a synthetic circuit.

**HUMAN PRACTICE**

- Bio-camp
- IJEM cooperation
- IJEM - Chinese website book
- Popularization on campus
- Popular science creation
- Surveys
- L-shaped folder

**CONTRIBUTION**

Ecofuel E.coli is a powerful design to generate isobutanol.

We have developed:
- Designed “Low temperature release” method
- Combined “Low temperature release system” with “zinc fingers”
- Maximum Yield of isobutanol up to 0.5 g/L
- Made isobutanol Producing BioBrick (BBa_KBB7002)
- Improved Isobutanol Producing BioBrick (BBa_KBB7000)
- DNA Program Correction (BBa_KBB7011)
- Produced instrument to increase our isobutanol production rate.