# The Oil Production of *Fusarium equiseti* UMN-1

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## The Oil Production of the fungal strain *Fusarium equiseti* UMN-1

The production of microbial lipids offers a potential utilization of lignocelluloses to generate biofuel products. While ethanol can only be blended with gasoline to reduce consumption, microbial lipids can be processed to “drop-in” fuel products and compatible to current engines.

Fungal lipid accumulation from lignocelluloses has great potential and deserves further research and development for the following reasons: 1) It is more suitable to develop a smaller scale and more robust bioconversion approach, which would be a significant advance in the bioenergy sciences & 2) It offers greater potential for lignocelluloses utilization and possible commercialization.

The objective of this research is to develop a small scale conversion platform to integrate agricultural biomass harvesting, packaging, storage, and pre-processing to produce fungal lipids as a “drop in” fuel.

## Research Summary

Research focused on the biomass production, lipid content, and oil content of the fungal strain Fusarium equiseti UMN-1. There were two primary research parts: Part 1 - study of the cultivation conditions and Part 2- Directed evolution of the Fusarium equiseti UMN-1 strain for enhanced cellulase production.

### Part 1 Study of cultivation conditions for the Fusarium equiseti UMN-1 strain

#### 1.1 Growth curve and lipid accumulation of the strain

the Fusarium equiseti strain was inoculated into a 100 mL liquid medium of 9.6 g/L potato dextrose and 12 g/L glucose in 250 mL flask. It was then cultivated at 27 ° C, 150 rpm for 2, 4, 5, 6, 7, 8, 10 days. Three replicates were prepared for each. The biomass content, lipid content, pH and residual sugar were tested for each replicate. The results are shown in Figure 1 and Table 1. From the plot shown in Figure 1 is can be determined that seven days is the optimal cultivation time for lipid production. At Day 6 the fungal dry biomass was at a reasonable stable level, and the lipid content began to decrease after Day 7.

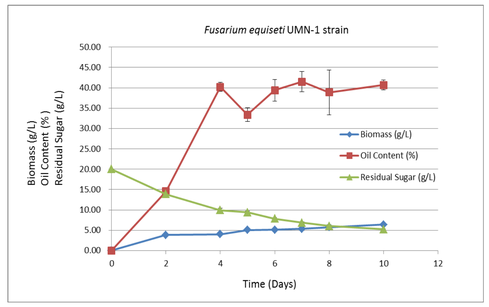


Figure 1. Growth curve and lipid accumulation of the *Fusarium equiseti* UMN-1 strain

| Days | pH |
|---|---|
| 0 | 6.33+/-0.00 |
| 2 | 5.86+/-0.06 |
| 4 | 5.93+/-0.03 |
| 5 | 6.08+/-0.06 |
| 6 | 5.78+/-0.04 |
| 7 | 5.03+/-0.07 |
| 8 | 6.02+/-0.04 |
| 10 | 6.03+/-0.06 |

Table 1. pH of the *Fusarium equiseti* UMN-1 strain

#### 1.2 Effect of temperature on lipid accumulation

The strain was inoculated into 100 mL liquid medium of 9.6 g/L potato dextrose and 12 g/L glucose in 250 mL flasks. It was cultivated at 150 rpm for 7 days at 20 ° C, 27 ° C, 35 ° C, and 42 ° C. Three replicates were made for each condition. Both the biomass and lipid content were analyzed. The analysis found that at 42 ° C growth ceased. At 35 ° C the greatest lipid content of 49.74+0.35%, and the greatest lipid production of was obtained at 27 ° C, with 0.219+0.019 g lipid/100 mL. Lipid production (lipid g/100 mL) is calculated by multiplying the biomass and lipid content. (See Figure 2 &3.)

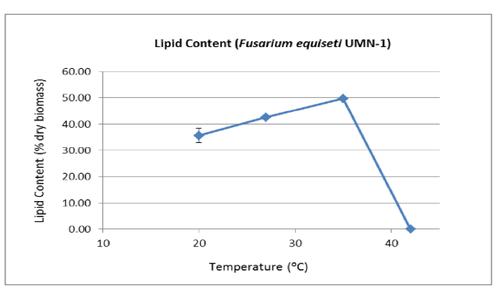


Figure 2. Effect of temperature on *Fusarium equiseti* UMN-1 strain lipid content

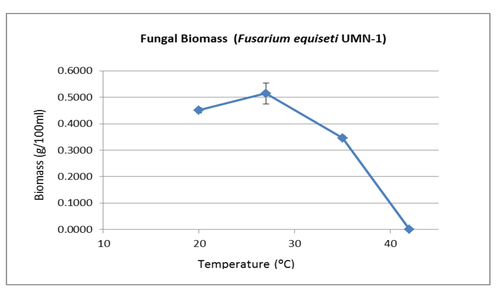


Figure 3. Effect of temperature on *Fusarium equiseti* UMN-1 strain lipid content

#### 1.3 Effect of agitation speed on lipid accumulation

The strain was inoculated into 100 mL liquid medium of 9.6 g/L potato dextrose and 12 g/L glucose in 250 mL flasks. It was cultivated at 27 ° C for 7 days at three different agitation speeds: 100 rpm, 150 rpm, 200 rpm. Three replicates were made for each condition. Each replicate was analyzed for both the biomass and lipid content. The analysis found the highest biomass content of 0.4987+0.0162 g/100 mL and highest lipid content of 44.47+1.78% was obtained at 150 rpm. The highest lipid production of 0.221+0.003 g lipid/100 mL was also obtained at 150 rpm (See Figure 4 & 5).

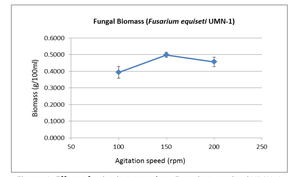


Figure 4. Effect of agitation speed on *Fusarium equiseti* UMN-1 strain biomass content

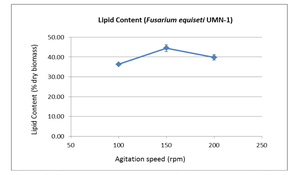


Figure 5. Effect of agitation speed on *Fusarium equiseti* UMN-1 strain lipid content

It was determined that the strain was able to form larger pellets at lower agitation speeds than at higher speeds. The typical observed results are shown in Figure 6. One can see there is no pellet formed at the higher speed of 200 rpm and the fungus grows in mycelium form.

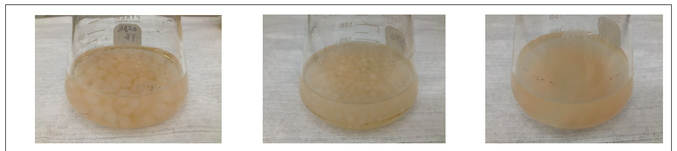


Figure 6. Pictures of *Fusarium equiseti* UMN-1 strain at three agitation speeds

### Part 2 Directed evolution of the Fusarium equiseti UMN-1 strain for enhanced cellulase production

The strain was cultivated in 100 mL medium at 150 rpm for 6 days at 27 ° C. The medium contained: 0.75 g/L (NH4)2SO4, 0.1 g/L MgSO4•7H2O, 1.4 g/L KH2PO4, 0.68 g/L K2HPO4, 0.1 g/L KCl, 0.8 mg/L MnSO4•H2O, 0.4 mg/L CuSO4, 0.8 mg/L FeSO4•7H2O, 0.8 mg/L Na2MoO4•2H2O, 8 mg/L ZnSO4•7H2O, 0.04 mg/L Na2B4O7 •10H2O, 0.75 g/L yeast extract, and either 20 g/L cellulose or 19 g/L cellulose+1 g/L xylose as a carbon source. After 6 days (and every subsequent 6 days), 10 mL of the culture broth was transferred into a new 100 mL medium. Ten generations of cultures were obtained by this continuous transfer in approximately 60 days. The remainder of each culture broth was centrifuged at 7000 rpm for 7 min. The mixture of fungal biomass and cellulose was extracted and dried at 105 ° C overnight. The lipid content of the solid was tested as the methods in Analytical method, but around 0.3 g dry solid was used. Lipid production (g/100 mL) is calculated by multiplying the solid and lipid content. In addition, the supernatants were collected to test cellulase activity (filter paper activity, FPA). Cellulase activity results were represented as filter paper unit per milliliter (FPU/mL).

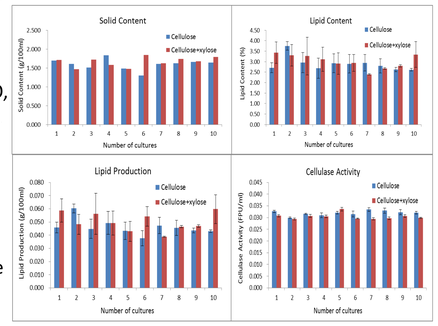


Figure 7. Solid Content, Lipid Content, Lipid Production, and Cellulase Activity of *Fusarium equiseti* UMN-1 strain

### *Analytical Methods*

***Lipid Content:***
*Lipids were extracted from the dry fungal biomass by using mixed solvents of chloroform and methanol with the following procedure (Folch J. et al., 1956): approximately 0.1 g of dry fungal biomass was added into a 10 mL mixture of chloroform and methanol (2:1 volume ratio of chloroform/methanol) and shaken at 150 rpm for 16 h. Then, 2.5 mL of water was added into the mixture and vortexed for 1 min, which was then centrifuged at 7,000 rpm for 7 min. After centrifugation, the lower layer was filtered through a 0.45 µm filter, and then the solvent was evaporated to obtain the total lipid. Lipid content was determined by calculating the weight percentage of lipid in dry biomass.*

***Sugar Content:***
*After cultivation, the culture broth was centrifuged at 7000 rpm for 7 min, and the supernatants were also collected to analyze residual sugar content in the fermentation broth using dinitrosalicylic acid, DNS method (Miller G.L., 1959).*

***Cellulase Activity:***
*The supernatants from the culture broth were collected and added as enzyme solution for cellulase activity by filter paper activity (FPA) assay (Ghose, T. K., 1987). Cellulase activity results were represented as filter paper unit per milliliter (FPU/mL). Filter Paper Activity Assay Method was used.*

## Summary and Discussion

**1.** Day 6 is the optimal cultivation time for lipid production. After Day 7, the lipid content begins to decrease.
**2.** The greatest lipid content of 49.74+0.35% was obtained at 35° C, and the greatest lipid production of 0.219+0.019 g lipid/100 mL was obtained at 27 ° C. At temperatures greater than 42° C fungal growth ceases.
**3.** At a rotation speed of 150 rpm the highest biomass content, lipid content, and lipid production was obtained.
4. Transference of the strain into a fresh medium does not significantly increase biomass content, lipid content, lipid production, or cellulase activity.

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