**Process Simulation and Techno-Economic Analysis of Large-Scale Bioproduction of Sweet Protein Thaumatin II**

Supplementary material

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**Transgenic Facilities Economic Inputs**

The facilities’ economic evaluation is based on the US dollar value in 2020. A 4% inflation rate is used to adjust for equipment purchase prices from previous years. Field growth economic variables were obtained from various sources. Fertilizer quantity and cost were obtained from a cost estimation spreadsheet developed University of Kentucky department of Agricultural Economics (UKAE) (TN-KY Burley Tobacco Budget, 2018). The quantity was estimated by linear extrapolation based on 120 days growth and adjusted from the 42 days growth period in this model. Field irrigation was estimated from the Food and Agriculture Organization of the United Nations water requirement for tobacco (Food and Agriculture Organization of the United Nations, n.d.). For land purchase prices, farm real estate average value per acre in Florida was estimated based on the USDA land values 2018 summary report (United States Department of National Agriculture (USDA) Agricultural Statistics Service, 2018). Drip irrigation costs were obtained from (Simonne et al., 2008). Fuel, lubrication, and repair costs for tractors and other field equipment were obtained from the 2015 UC ANR field cost study (Smith & Tumber, 2015) and were adjusted based on the average annual spinach producer price indices obtained from the Federal Reserve Bank of St. Louis (FRED Federal Reserve Bank of St. Louis, 2020). Indoor cost variables were adapted from (McNulty et al., 2019). Downstream processing economic values were obtained from (Nandi et al., 2016), (McNulty et al., 2019), SuperPro Designer default values, and WPK. Startup and validation costs were estimated as 5% of direct fixed capital (DFC). Working capital was estimated to cover expenses for 30 days of operation.

**Transient Facility Economic Inputs**

The facility’s economic evaluation is based on the US dollar value in 2020. A 4% inflation rate is used to adjust for equipment purchase prices from previous years. Spinach field growth economic parameters were adjusted based on the average annual spinach producer price indices obtained from the Federal Reserve Bank of St. Louis (FRED Federal Reserve Bank of St. Louis, 2020). VPL’s economic parameters were adapted from (Nandi et al., 2016) and equipment purchase prices were adjusted according to the following equation:

$$C=C\_{0}\left(\frac{Q}{Q\_{0}}\right)^{0.6}$$

where C is the equipment cost, C0 is the base cost, Q is the capacity variable, Q0 is the base capacity. Other unit operation equipment costs were estimated from the built-in SuperPro Designer cost models.

Farm real estate average value per acre in California was estimated based on the USDA land values 2018 summary report (USDA, 2018). Drip irrigation costs were obtained from Simonne et al. (2008). Fuel, lubrication, and repair costs for tractors and other field equipment were obtained from the 2015 UC ANR cost study (Smith & Tumber, 2015). Downstream processing economic values were obtained from Nandi et al. (2016), McNulty et al. (2019), SuperPro Designer default values, and WPK.

**Table S1**. Transgenic thaumatin production facilities base case design parameters and assumptions; FW, fresh weight; WPK, working process knowledge; Calc, calculation; MT, metric ton

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Value** | **Unit** | **Reference** |
| **Upstream facility (field), with downstream chromatography** |
| Production level | 50 | MT thaumatin/yr | Assumption |
| Number of batches | 157 | batches/yr | Calc |
| Batch duration  | 45.3 | days | Calc |
| Recipe cycle time | 2 | days | Calc |
| Growth time (seeding to induction) | 35 | days | WPK |
| Incubation time (induction to harvest) | 7 | days | WPK |
| Land turnaround duration | 3 | days | WPK |
| Thaumatin expression level | 1.5 | g/kg FW | WPK |
| Plant density | 130,000 | plants/acre | Assumption(based on 3 plants/ft2) |
| N. tabacum aerial biomass at harvest | 100 | g/plant | [(Knödler, Rühl, Emonts, & Buyel, 2019)] |
| Germination efficiency | 90 | % | Assumption |
| Acreage per batch | 24.5 | acres | Calc |
| Number of plots | 22 | plots/total field | Calc |
| Total field acreage (footprint) | 538 | acres | Calc |
| Total annual cultivated acreage  | 3,850 | acres/yr | Calc |
| Location | Florida, USA |  | Assumption |
| **Upstream facility (indoor), with downstream chromatography** |
| Production level | 50 | MT thaumatin/yr |  |
| Number of batches | 157 | batches/yr | Calc |
| Batch duration  | 42.6 | days | Calc |
| Recipe cycle time | 2 | days | Calc |
| Growth time (seeding-induction) | 35 | days | [(McNulty et al., 2019)] |
| Incubation time (induction-harvest) | 7 | days | [(McNulty et al., 2019)] |
| Thaumatin expression level | 1.5 | g/kg FW | WPK |
| N. benthamiana aerial biomass at harvest  | 15 | g/plant | [(McNulty et al., 2019)] |
| Plants per batch | 21,200,000 | plants/batch | Calc |
| Germination efficiency  | 95 | % | Assumption |
| Plants per tray | 94 | Plants tray | [(McNulty et al., 2019)] |
| Tray area | 0.15 | m2/tray | [(McNulty et al., 2019)] |
| Growth space design | 10 | layers | Assumption |
| Growth space utilization | 90 | % | Assumption |
| Facility footprint  | 83,000 | m2 | Calc |
| **Downstream processing facility, with chromatography** |
| Batch duration | 54.5 | hours | Calc |
| Downstream recovery | 66.8 | % | Assumption |
| Final product purity | 98.0 | % | Assumption |
| **Downstream processing facility, without chromatography** |
| Batch duration | 38.4 | hours | Calc |
| Downstream recovery | 80 | % | Assumption |
| Final product purity | 74.8 | % | Assumption |

**Table S2**. Transient production of thaumatin in spinach base case parameters and assumptions FW, fresh weight; WPK, working process knowledge; Calc, calculation; MT, metric ton

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Value** | **Unit** | **Reference** |
| **Overall facility** |
| Production level | 50 | MT thaumatin/yr |  |
| Number of batches | 153 | batches/yr | Calc |
| Batch duration  | 68 | days | Calc |
| Recipe cycle time | 1.94 | days | Calc |
| Location | California, USA |  |  |
| **Spinach field growth** |
| Growth time (seeding-spraying) | 45  | days | [(Koike et al., 2011)] |
| Incubation time (spraying-harvest) | 15 | days | WPK |
| Thaumatin expression level | 1 | g/kg FW | WPK |
| Field plant density | 174,240 | plants/acre | Assumption |
| Spinach yield | 15,240 | kg FW/acre | [(Koike et al., 2011)], WPK |
| Seed quantity | 1.25 million | seeds/acre | [(Koike et al., 2011),(Smith & Tumber, 2015)] |
| 31.3 | lbs/acre |
| Acreage per batch  | 22.6 | acres/batch | Calc |
| Number of plots | 34 | plots/total field | Calc |
| Total field acreage (footprint) | 767 | acres | Calc |
| Total cultivated acreage (assuming no reusing of land) | 3,450 | acres | Calc |
| **Viral particles production**  |
| N. benthamiana growth time (seeding-infiltration) | 35 | days | [(Nandi et al., 2016)] |
| N. benthamiana incubation time (infiltration-harvest) | 7 | days | [(Nandi et al., 2016)] |
| Viral particles expression level | 1 | g/kg FW | [(Klimyuk, Pogue, Herz, Butler, & Haydon, 2014)] |
| Viral particle concentration in spray suspension | 1014 | particles/L | WPK |
| Viral particle molecular weight | 31,750 | kDa | WPK |
| Spray volume requirement | 2 | mL/plant | WPK |
| **Downstream Processing** |
| Downstream recovery | 95 | % | WPK |
| Downstream Processing time | 30.2 | hrs/batch | Calc |
| Final thaumatin purity | 94 | % | Assumption |

**Table S3**. Downstream processing losses breakdown per unit operation; P&F, plate and frame filtration; DSP, downstream processing; UF/DF, ultrafiltration/diafiltration; Chrom, chromatography

|  |
| --- |
| **DSP facility without chromatography** |
| Step | Screw Press | P&F 1 | P&F 2 | P&F 3 | UF/DF | Chromand UF/DF 2 | Drying |
| Loss (% of initial thaumatin) | 3.0 | 5.0 | 5.0 | 1.5 | 5.0 | - | 0.5 |
| Cumulative recovery (% of initial thaumatin) | 97.0 | 92.0 | 87.0 | 85.5 | 80.5 | - | 80.0 |
| Start (kg/batch) | 398 | 386 | 366 | 346 | 340 | - | 320 |
| End (kg/batch) | 386 | 366 | 346 | 340 | 320 | - | 319 |
| % loss per unit | 3.0 | 5.2 | 5.4 | 1.7 | 5.8 | - | 0.6 |
| **DSP facility with chromatography** |
| Step | Screw Press | P&F 1 | P&F 2 | P&F 3 | UF/DF | Chromand UF/DF 2 | Drying |
| Loss (% of initial thaumatin) | 3.0 | 5.0 | 5.0 | 1.5 | 5.0 | 13.2 | 0.5 |
| Cumulative recovery (% of initial thaumatin) | 97.0 | 92.0 | 87.0 | 85.5 | 80.5 | 67.3 | 66.8 |
| Start (kg/batch) | 477 | 463 | 439 | 415 | 408 | 384 | 320 |
| End (kg/batch) | 463 | 439 | 415 | 408 | 384 | 320 | 319 |
| % loss per unit | 3.0 | 5.2 | 5.4 | 1.7 | 5.8 | 17 | 0.6 |

|  |  |  |  |
| --- | --- | --- | --- |
|  | Upstream (Field) | Upstream (Indoor) | Downstream |
| Unlisted Equipment | Seeding:0.03 x PC\* | Seeding:0.2 x PC | Entire Facility:0.2 x PC |
| Plant Growth:0.03 x PC | Plant Growth:0.2 x PC |
| Induction + Incubation:0.03 x PC | Induction + Incubation:0.2 x PC |
| Harvesting:0.03 x PC | Harvesting0.2 x PC |
| Transportation:0.2 x PC | Transportation0.2 x PC |
| Lang Factor | Seeding:1.0 x PC | Seeding:3.0 x PC | Entire Facility:DFC= DC+IC+OC |
| Plant Growth:1.0 x PC | Plant Growth:3.0 x PC | DC: \*\*Piping (A)= 0.35 x PCInstrumentation (B)= 0.40 x PCInsulation (C)= 0.03 x PCElectrical Facilities (D)= 0.10 x PCBuildings (E)= 0.45 x PCYard Improvement (F)= 0.15 x PCAuxiliary Facilities (G)= 0.40 x PC |
| Induction + Incubation:1.0 x PC | Induction + Incubation:3.0 x PC | Unlisted Equipment Installation Cost= 0.50 x Unlisted Equipment purchase costListed Equipment Installation Cost: Equipment specific |
| Harvesting:1.0 x PC | Harvesting:3.0 x PC | IC:Engineering= 0.25 x DCConstruction= 0.25 x DC |
| Transportation:3.0 x PC | Transportation:3.0 x PC | OC:Contractor’s Fee= 0.05 x (DC + IC)Contingency= 0.10 x (DC + IC) |

**Table S4**. Transgenic production facilities DFC estimation parameters. DFC, direct fixed cost; PC, purchase cost; DC, direct cost; IC, indirect cost; OC, other costs.

\*Purchase Cost (PC) = Listed equipment purchase cost + unlisted equipment purchase cost

\*\* Direct Cost (DC)= PC + Installation +A + B + C + D + E + F + G

|  |  |  |  |
| --- | --- | --- | --- |
|  | VPL | Field Growth | Downstream |
| Unlisted Equipment | 0.2 x PC | 0.03 x PC | Entire Facility:0.2 x PC |
| Lang Factor | 3.0 x PC | 1.0 x PC | Entire Facility:DFC= DC+IC+OC |
| DC: \*\*Piping (A)= 0.35 x PCInstrumentation (B)= 0.40 x PCInsulation (C)= 0.03 x PCElectrical Facilities (D)= 0.10 x PCBuildings (E)= 0.45 x PCYard Improvement (F)= 0.15 x PCAuxiliary Facilities (G)= 0.40 x PC |
| Unlisted Equipment Installation Cost= 0.50 x Unlisted Equipment purchase costListed Equipment Installation Cost: Equipment specific |
| IC:Engineering= 0.25 x DCConstruction= 0.25 x DC |
| OC:Contractor’s Fee= 0.05 x (DC + IC)Contingency= 0.10 x (DC + IC) |

**Table S5**. Transient production facility DFC estimation parameters. DFC, direct fixed cost; PC, purchase cost; DC, direct cost; IC, indirect cost; OC, other costs; VPL, virion production laboratory.

\*Purchase Cost (PC) = Listed equipment purchase cost + unlisted equipment purchase cost

\*\* Direct Cost (DC)= PC + Installation +A + B + C + D + E + F + G

**Table S6**. Working capital (WC) estimation parameters for all facilities.

|  |  |
| --- | --- |
| Parameter | Value |
| **Cover labor expenses for** | 30 days |
| **Cover raw materials expenses for**  | 30 days |
| **Cover utilities expenses for**  | 30 days |
| **Cover waste treatment expenses for**  | 30 days |
| **Startup and Validation** | 5% of DFC |

**Table S7**. Transgenic production facilities detailed annual labor cost. BLC, basic labor cost; TLC, total labor cost.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Facility | Labor type | BLC | TLC\*\*\* | Direct Demand Hours per year | Total Demand Hours per year |
| **Upstream (field)** | Upstream operator | $17/h | $39.10/h | 30,647 | 40,863 |
| **Upstream (Indoor)** | Upstream operator | $20/h | $46/h | 3,938 | 4,145  |
| **Downstream** | Downstream operator | $25/h | $57.50/h | 21,663 | 28,884  |

\*\*\*TLC= BLC x (1 + Benefits (0.4) + Supervision (0.2) + Supplies (0.1) + Administration (0.6))

**Table S8**. Transient production facility detailed annual labor cost. BLC, basic labor cost; TLC, total labor cost.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| FacilitySection | Labor type | BLC | TLC\*\*\* | Direct Demand Hours per year | Total Demand Hours per year |
| **VLP** | Upstream operator | $20/h | $46/h | 13,616 | 18,155 |
| **Field Growth** | Field operator | $17/h | $39.10/h | 36,620 | 48,827 |
| **Downstream** | Downstream operator | $25/h | $57.50/h | 7,919 | 10,559  |

\*\*\*TLC= BLC x (1 + Benefits (0.4) + Supervision (0.2) + Supplies (0.1) + Administration (0.6))

**Table S9**. Transgenic production facilities dependent costs estimation parameters

|  |  |  |
| --- | --- | --- |
| Facility | Raw Material | Unit Cost |
| Upstream (field) | Maintenance | Included as consumables |
| Depreciation | Straight line over 10 years (5 % salvage value). Land is non-depreciable. |
| Insurance | 0.09% DFC |
| Local taxes | 2.51% DFC |
| Factory expenses | 0.12% DFC |
| Upstream (indoor) | Maintenance | Section dependent (0.10-0.40 % DFC) |
| Depreciation | Straight line over 10 years (5 % salvage value) |
| Insurance | 1% DFC |
| Local taxes | 2% DFC |
| Factory expenses | 5% DFC |
| Downstream | Maintenance | Equipment specific  |
| Depreciation | Straight line over 20 years (5 % salvage value) |
| Insurance | 1% DFC |
| Local taxes | 2% DFC |
| Factory expenses | 5% DFC |

**Table S10.** Transient production facilities dependent costs estimation parameters

|  |  |  |
| --- | --- | --- |
| Facility | Raw Material | Unit Cost |
| VPL | Maintenance | 0.40 % DFC |
| Depreciation | Straight line over 10 years (5 % salvage value). Land is non-depreciable. |
| Insurance | 0.09% DFC |
| Local taxes | 2.51% DFC |
| Factory expenses | 0.12% DFC |
| Field growth | Maintenance | Included as consumables |
| Depreciation | Straight line over 10 years (5 % salvage value) |
| Insurance | 0.09% DFC |
| Local taxes | 2.51% DFC |
| Factory expenses | 0.12% DFC |
| Downstream | Maintenance | Equipment specific  |
| Depreciation | Straight line over 10 years (5 % salvage value) |
| Insurance | 1% DFC |
| Local taxes | 2% DFC |
| Factory expenses | 5% DFC |



**Figure S1**. SuperPro Designer model flowsheet for vertical farming (indoor) upstream transgenic production facility



**Figure S2**. SuperPro Designer model flowsheet for thaumatin transient production in spinach. V-103: 73,000L (10 in parallel)

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