# ENZYMATIC HYDROLYSIS OF RECYCLED WASTE FINES

BY

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# Objective

- Optimization of reaction kinetics of enzymatic hydrolysis of recycled paper mill waste fines, predict the competitive adsorptive reactions of enzymes & surfactants with mineral particles (Mathematical Modeling)

- Propose  a Response Surface Model (RSM) for analyzing scale up

- Analyze &  predict optimum design & operating conditions  for plants of various throughputs, related economics & environmental impacts

# Introduction

- Paper mills using recycled pulp produce significant solid waste
- Added chemicals &  starch are disadvantages for waste water (BOD), landfill & disposal cost burden to the mill
- Contents - Cellulose, hemi-cellulose from fines/fibers- can be converted to useful sugar
- 14 Million tons/year of potential cellulose in USA can be converted to sugar (huge savings); otherwise solid waste (Approx. $625 million to landfill from about 170 mills in USA)

# Fines in Recycled Paper

**Fibers must bend and drape**

- Good bonding and give strength to paper – essential quality of packaging paper grades

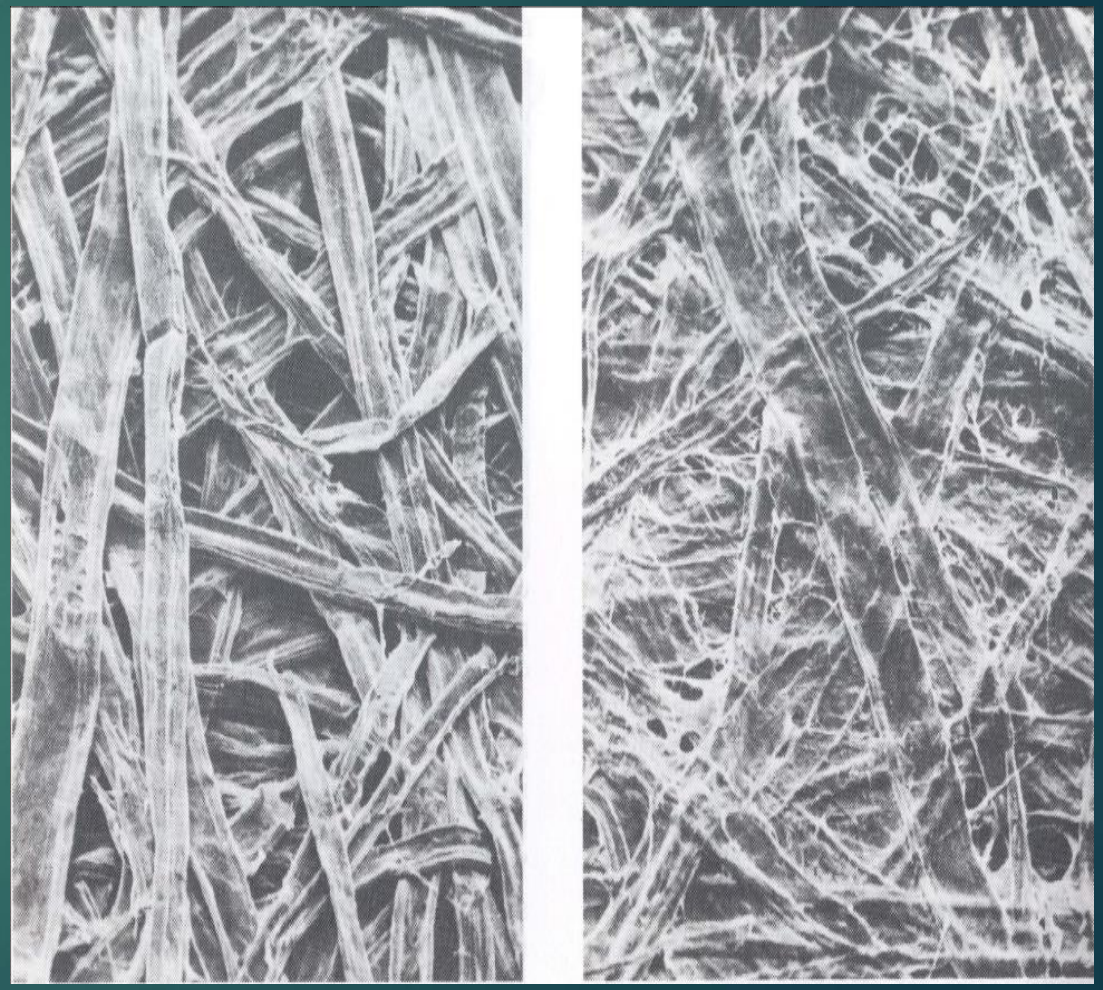
**Recycling causes hornification**

- Due to repeated drying and wetting cycles
- Increased crystallinity leads to stiffer and more brittle fibers

**Pulp Refining to increase bonding**

- Due to repeated drying and wetting cycles
- Increased crystallinity leads to stiffer and more brittle fibers

**Recycled fibers do not refine – break into fines**



# Fines in Recycled Paper

**Recycled Fines**

- Do not swell
- Hard
- Increase sheet bulk
- Interfere with bonding

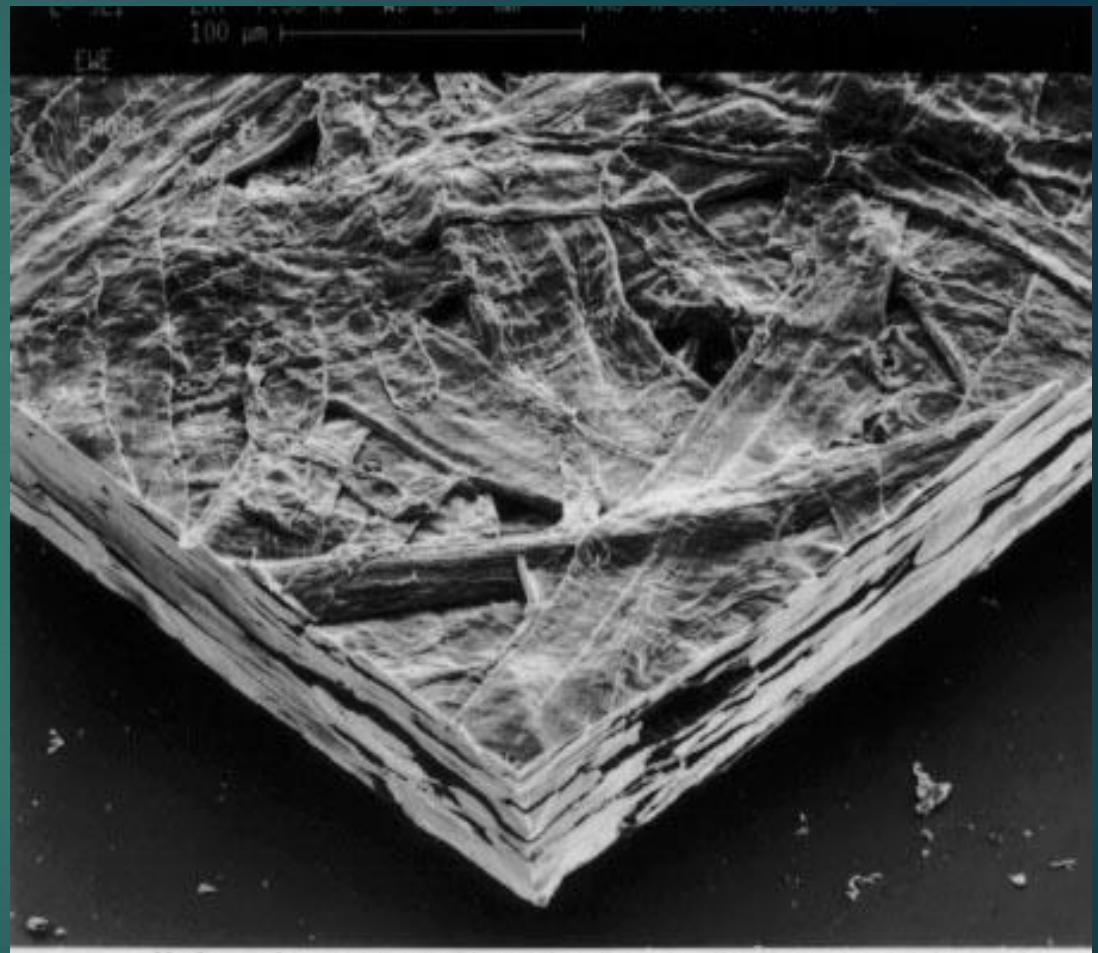
**Represent valuable stock paid for**

**Cannot afford to lose them**

**Waste ends up in effluent**

- **Increased solid waste**
- **Increased BOD**

**Solution – try to carry them in the sheet**



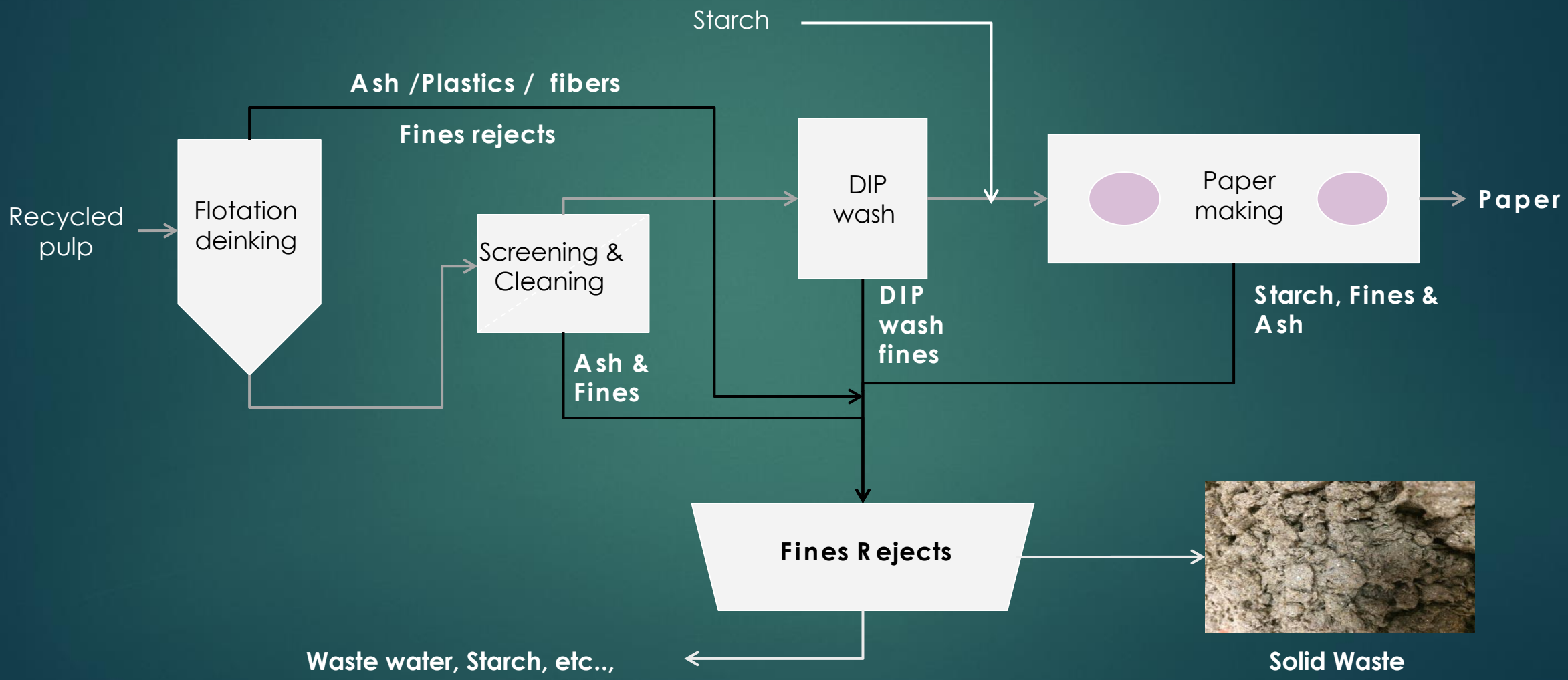
Hydrocyclone separated Longview Douglas fir - Earlywood enhanced

# High Ash contents

- Ash
  - Minerals from coating & fillers
  - Kaolin
  - $CaCO_3$



# Paper Mill Fines & Fiber Rejects



# Problems caused by fines

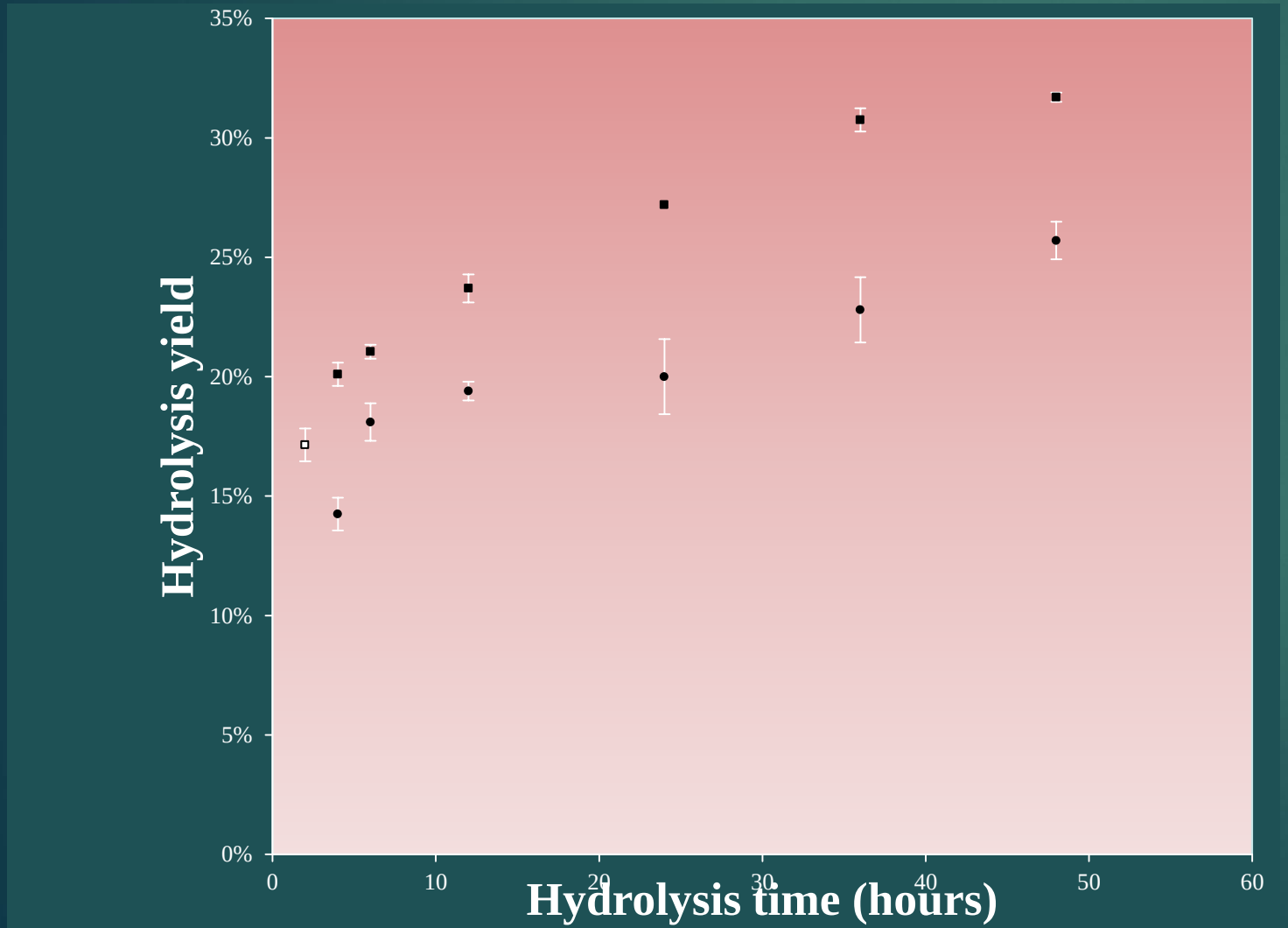
- Reduce sheet quality (bonding, strength)
- Interfere with drainage
- Interfere with drying
- Reduce production rate
- Cant remove them in current process

# Literature Survey – Physical & chemical characteristics of waste fines from a recycled linerboard mill (OCC)

| Fines | Value |
|---|---|
| Cellulose | 60% |
| Hemicellulose | > 5% |
| Lignin | > 3% |
| Total Ash Content | 33 % (by mass) |
| Calcium Carbonate | 15% |
| Kaolin | 18 % |
| Zeta Potential | -9 mV |
| pH | 6.4 |
| Particle Size | 2.1 – 3 µm |

Opportunity to use fines based cellulose in lieu of plant based

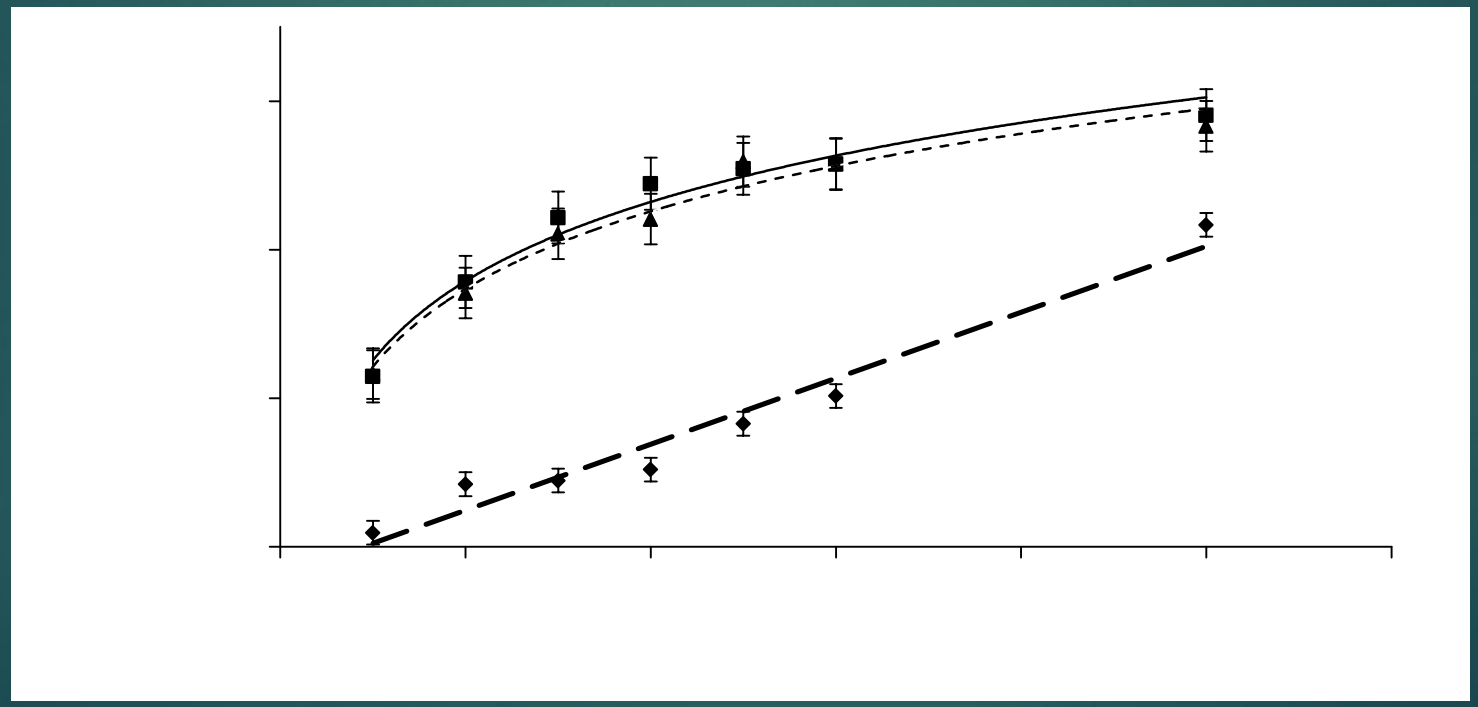
# Hydrolysis kinetics of Avicel (■) and fines (●)




- Kinetics of sugar production:
  - Yields are lower (on % hydrolyzable sugar basis)
  - Slower kinetics
  - Importance of inhibition
    - (Min et. al, 2014-17)

# Filler effect

- $CaCO_3$ and Kaolin
  - Addition $CaCO_3$ or Kaolin (30%) to UKP
    - $CaCO_3$: High inhibitory potential
    - Kaolin: Low inhibitory potential



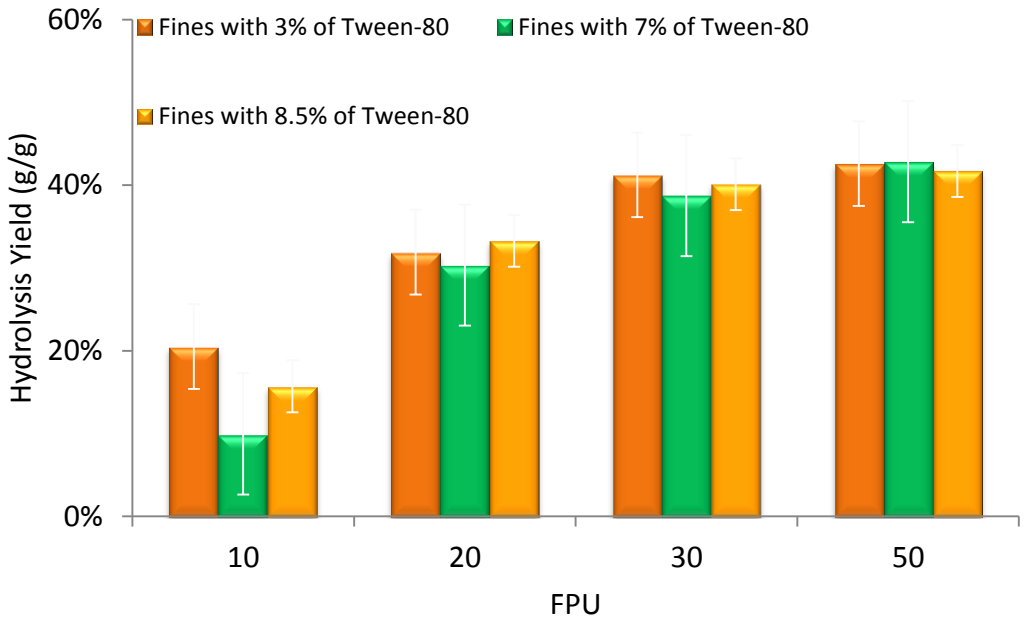
Effect of surfactant (3% dosage per 1 g of fines) on the enzymatic hydrolysis yield. Enzyme loading 20 FPU/g (OD fines).

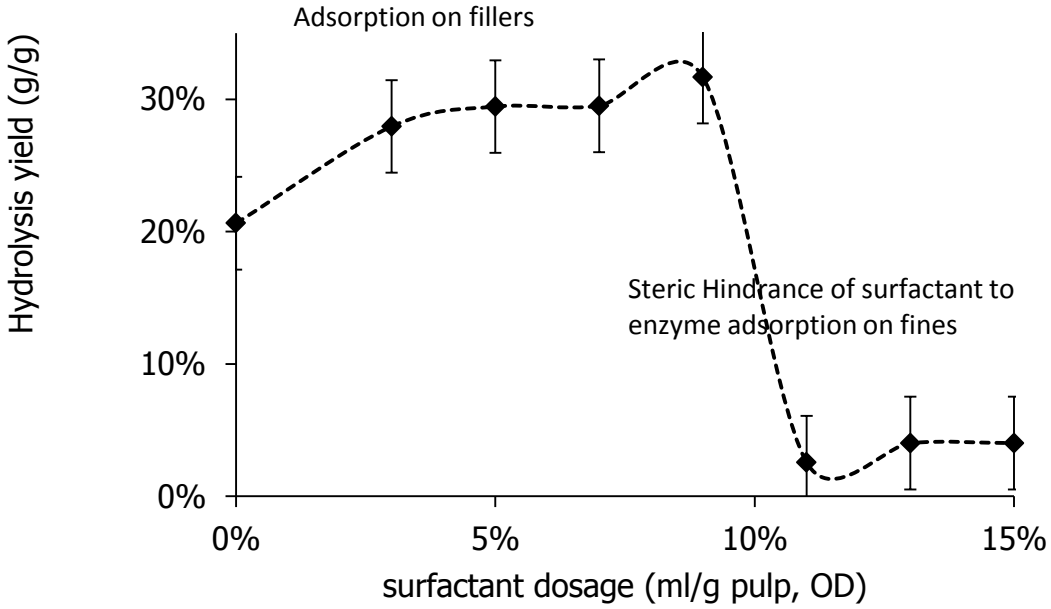
| Surfactants | None | Anionic (SDS) | Cationic (CTAB) | Non-ionic (Tween-80) |
|---|---|---|---|---|
| **Hydrolysis yield (%)** | 20 (±3.3) | 19 (±2.4) | 20 (±4.1) | 33 (±3.1) |

Numbers in parenthesis give the standard deviation.

Effects of fillers (kaolin and calcium carbonate) and nonionic surfactant (Tween 80) on enzymatic (10 FPU, 72 hours) hydr olysis yield of artificial fines mixture with milled UKP (softwo od, 60 mesh) and fillers.

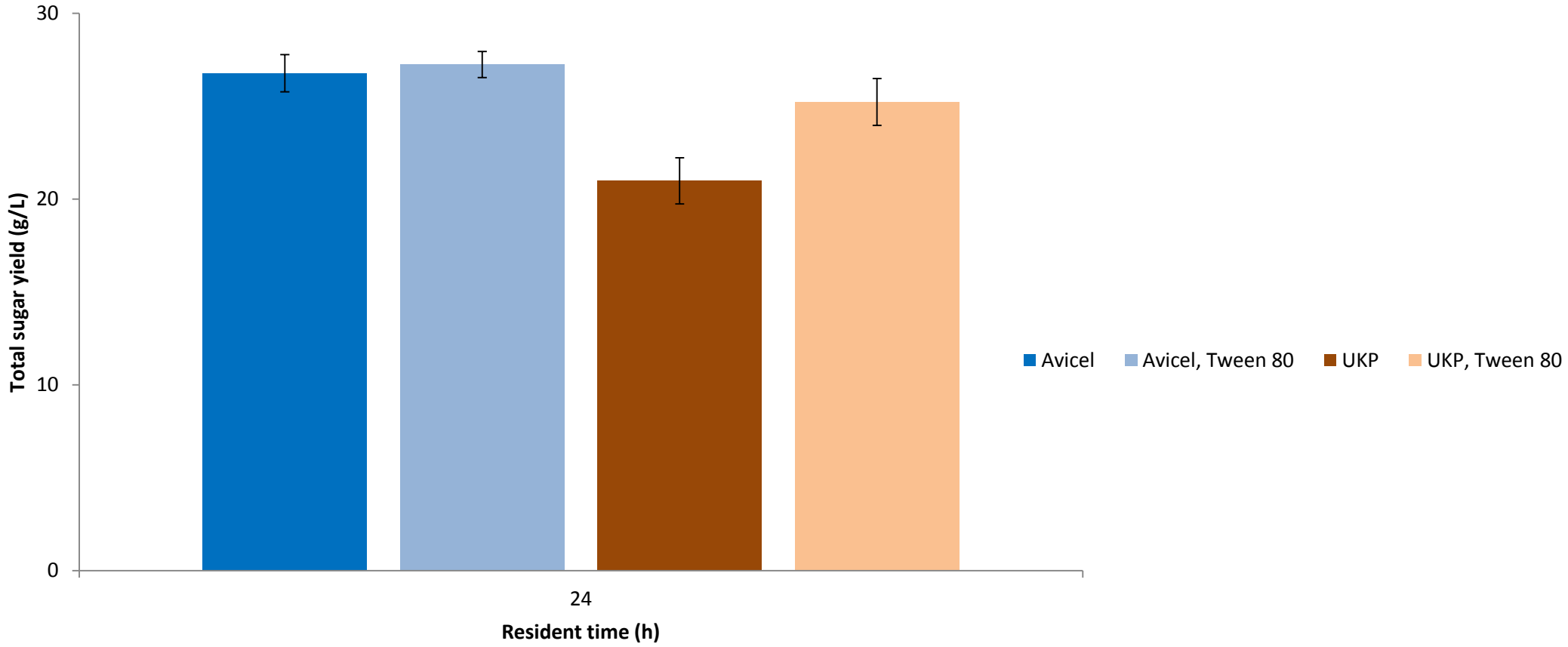
- **Fines**
  - **Dosage from 3% to 9% = increase yield**
  - **Exceeded 10% dosage = negative effect**



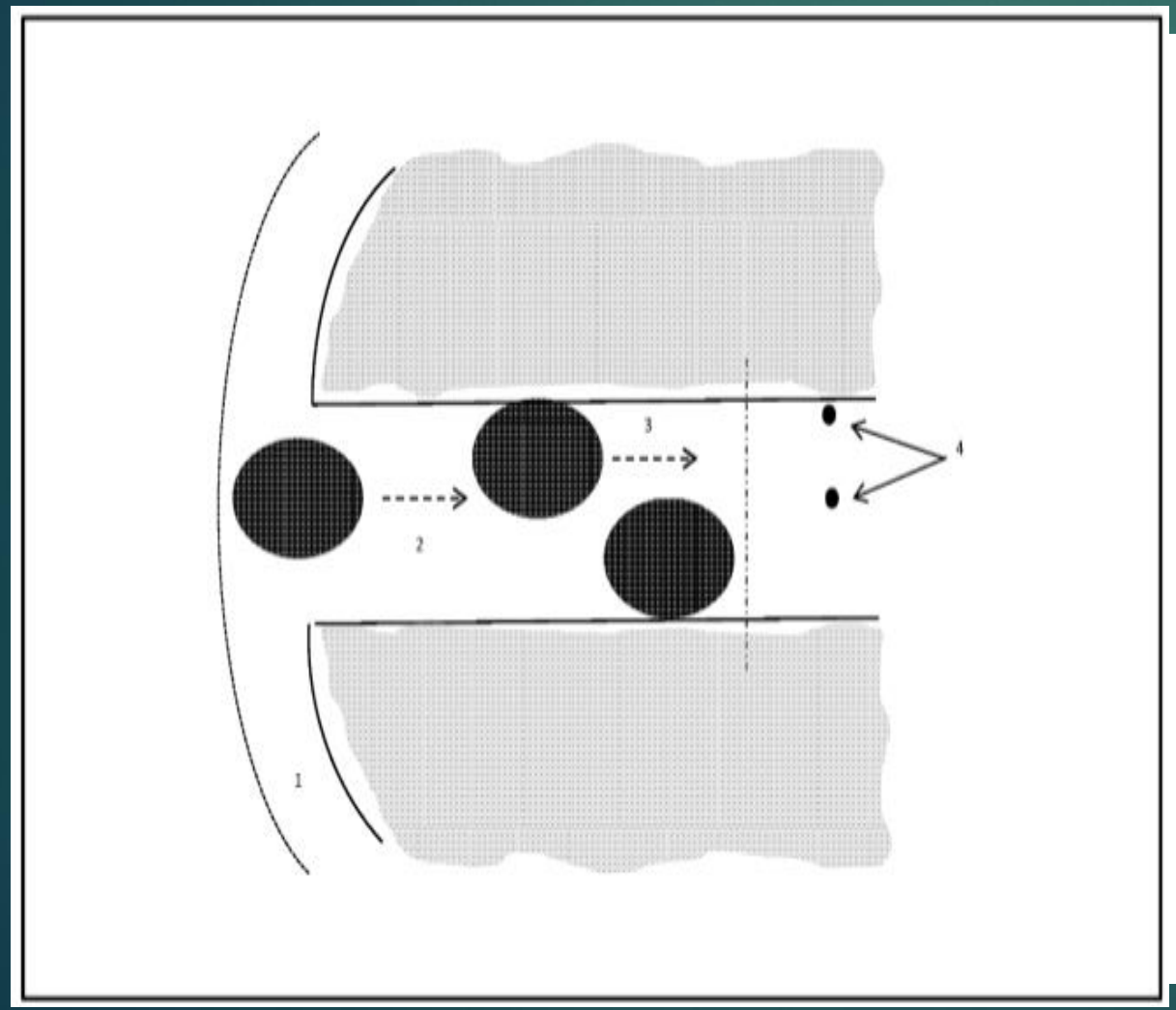




Hydrolysis yield of fines depending on different Tween 80 dosage with 20 FPU of *T. reesei* cellulase for 72hours.

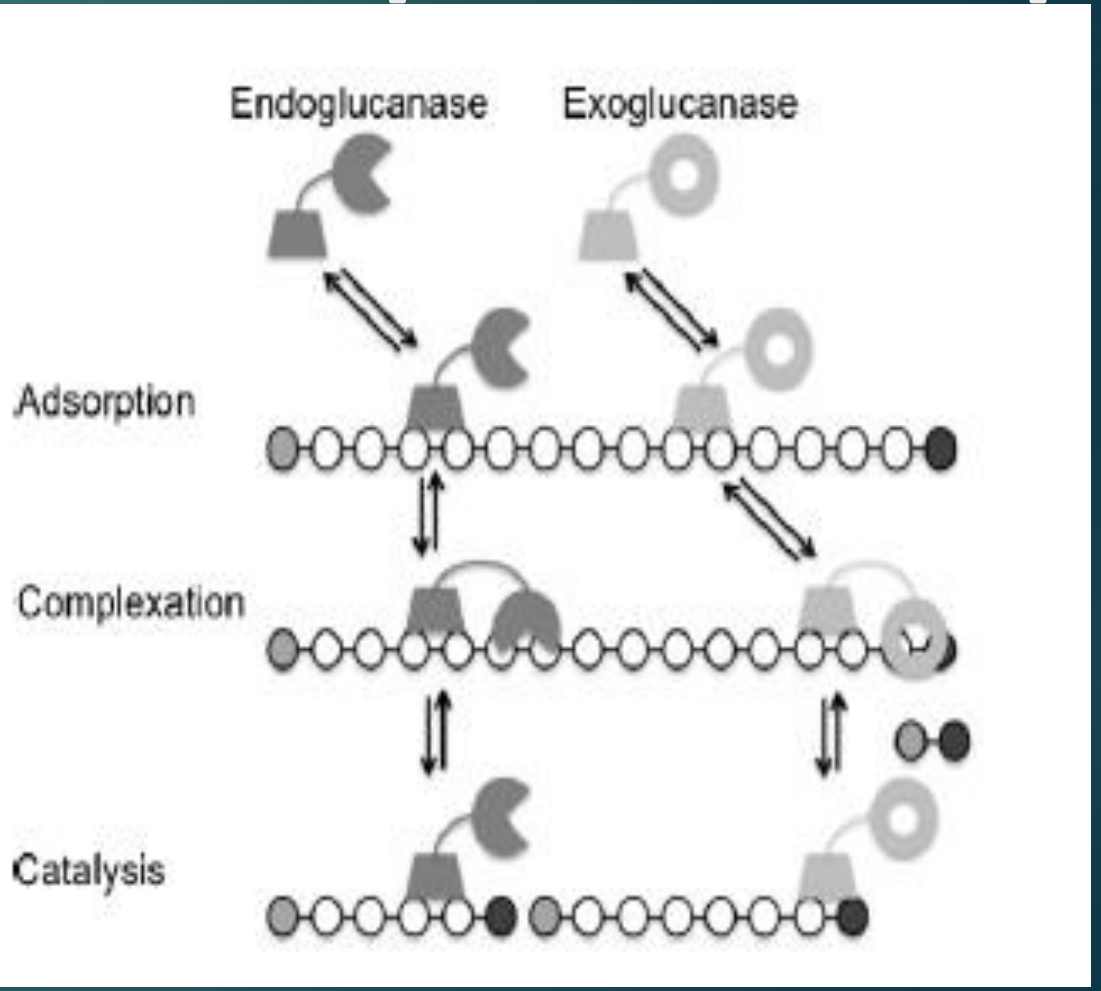
# Surfactant improves hydrolysis of fibers too



# Mathematical Modeling - Enzyme Transport Process




**Stages Of Enzymatic Hydrolysis Of Cellulose Chains. [Levine et al. 2010].**



# Kinetic Model: Partial Differential Equations
# Modified model from Luterbacher et al., 2013a-c

$$\varepsilon \frac{\partial c}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 D_p \frac{\partial c}{\partial r}\right) - \rho G \qquad (1)$$

For adsorbed phase $0 < r < R \quad t > 0$

$$\frac{\partial q}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2 D_q \frac{\partial q}{\partial r}\right) + G \qquad (2)$$

- **Simplified model eliminating diffusional resistances**

# Mathematical Modeling - Hydrolysis Model for Cellulose

$$E + S \underset{K_{2S}}{\overset{K_{1S}}{\rightleftharpoons}} E^*S \qquad (1)$$

$$E^*S \xrightarrow{K_{3P}} E + P \qquad (2)$$

$$E + I \underset{K_{2I}}{\overset{K_{1I}}{\rightleftharpoons}} E^*\mathrm{I} \qquad (3)$$

$$E + P \underset{K_{2p}}{\overset{K_{1p}}{\rightleftharpoons}} E^*P \qquad (4)$$

Four (4) reactions modeled so far.

- Adsorption onto cellulose substrate (Langmuir kinetics)
- Dissociation of S into product sugars P
- Adsorption by CC (Mineral Surfaces) – Inactivation of enzyme
- Product inhibition (sugar in solution inactivating the enzyme)

# Mathematical Modeling

We Introduce:

$$S_u + I \underset{K_{4I}}{\overset{K_{3I}}{\rightleftharpoons}} S_u{}^{*} I \qquad (5)$$

**Surfactant**

- **Adsorption onto CC (competitive with Langmuir/Enzyme kinetics)**

# Mathematical Modeling - Kinetic Reaction Rates:

The kinetic equation describing the change in concentration of the enzyme-cellulose complex reactions (1) and (2).

$$\frac{dq_1}{dt} = k_{1S}cq_2 - (k_{2S} + k_{3P})q_1 \qquad (6)$$

$$\frac{dq_2}{dt} = k_{2S}q_1 + \sigma k_{3P}q_1 - k_{1S}cq_2 \qquad (7)$$

| Symbol | Variable |
|---|---|
| $q_1$ | Concentration of enzyme-cellulose complex on fine particle surface, g/L (solution) |
| $q_2$ | Concentration of cellulose (fine particles), g/L (solution) |
| $k_{1S}$ | Forward Rate Constant |
| $k_{2S}$ | Backward Rate Constant |
| $k_{3P}$ | Product Rate Constant |
| $\sigma$ | negative due to loss of surface area of cellulose for adsorption (fitted to experimental data) |
| $c$ | Concentration of enzyme |

# Mathematical Modelling

The kinetic equation for the enzyme sugar complex due to product inhibition

$$\frac{dq_3}{dt} = k_{1P}cq_4 - k_{2P}q_3 \qquad (8)$$

The concentration of the monomeric sugar (product)

$$\frac{dq_4}{dt} = k_{3P}q_1 + k_{2P}q_3 - k_{1P}cq_4 \qquad (9)$$

| Symbol | Variable |
|---|---|
| $q_3$ | Concentration of enzyme-sugar complex due to product inhibition, g/L (solution) |
| $q_4$ | Concentration of monomer sugar products from cellulose in fine particles, g/L (solution) |
| $k_{1p}$ | Forward Sugar Product Rate Constant |
| $k_{2p}$ | Backward Sugar Product Rate Constant |
| $k_{3P}$ | Rate Constant for product formation |

# Mathematical Modeling

The kinetics of enzyme and surfactant adsorption on the fillers i.e. reactions (3) and (5) are given below

$$\frac{dq_5}{dt} = k_{1I} c q_I - k_{2I} q_5 \qquad (10)$$

$$\frac{dq_6}{dt} = k_{3I} c_{Su} q_I - k_{4I} q_6 \qquad (11)$$

| Symbol | Variable |
|---|---|
| $q_5$ | Concentration of enzyme-inhibitor complex on filler CC surface, g/L (solution) |
| $q_6$ | Concentration of surfactant-inhibitor complex on filler CC surface, g/L (solution) |
| $k_{1I}$ | Rate Constant |
| $k_{2I}$ | Rate Constant |
| $k_{3I}$ | Rate Constant |
| $k_{4I}$ | Rate Constant |
| $q_I$ | Concentration of inhibitor, g/L (solution) |

# Mathematical Modelling

Enzyme mass balance

| | |
|---|---|
| $\frac{dc}{dt} + \frac{dq_1}{dt} + \frac{dq_3}{dt} + \frac{dq_5}{dt} = 0$ | **( 12)** |

Integration, Initial concentration $c_0$.

| | |
|---|---|
| $c = c_0 - (q_1 + q_3 + q_5)$ | **( 13)** |

# Mathematical Modeling

## Surfactant Mass Balance

| | |
|---|---|
| $c_{Su} = c_{Su,0} - (q_6)$ | **(14)** |

Enzyme and surfactant are adsorbed competitively onto the CC.

| | |
|---|---|
| $q_I = q_m - (\mathbf{a}q_5 + \mathbf{b}q_6)$ | **(15)** |

Where,

$\mathbf{q_m}$ = Filler surface

a & b = empirical constants, assume = 1

Solved using Runge-Kutta Method

# Mathematical Modeling - Example Model Calculation

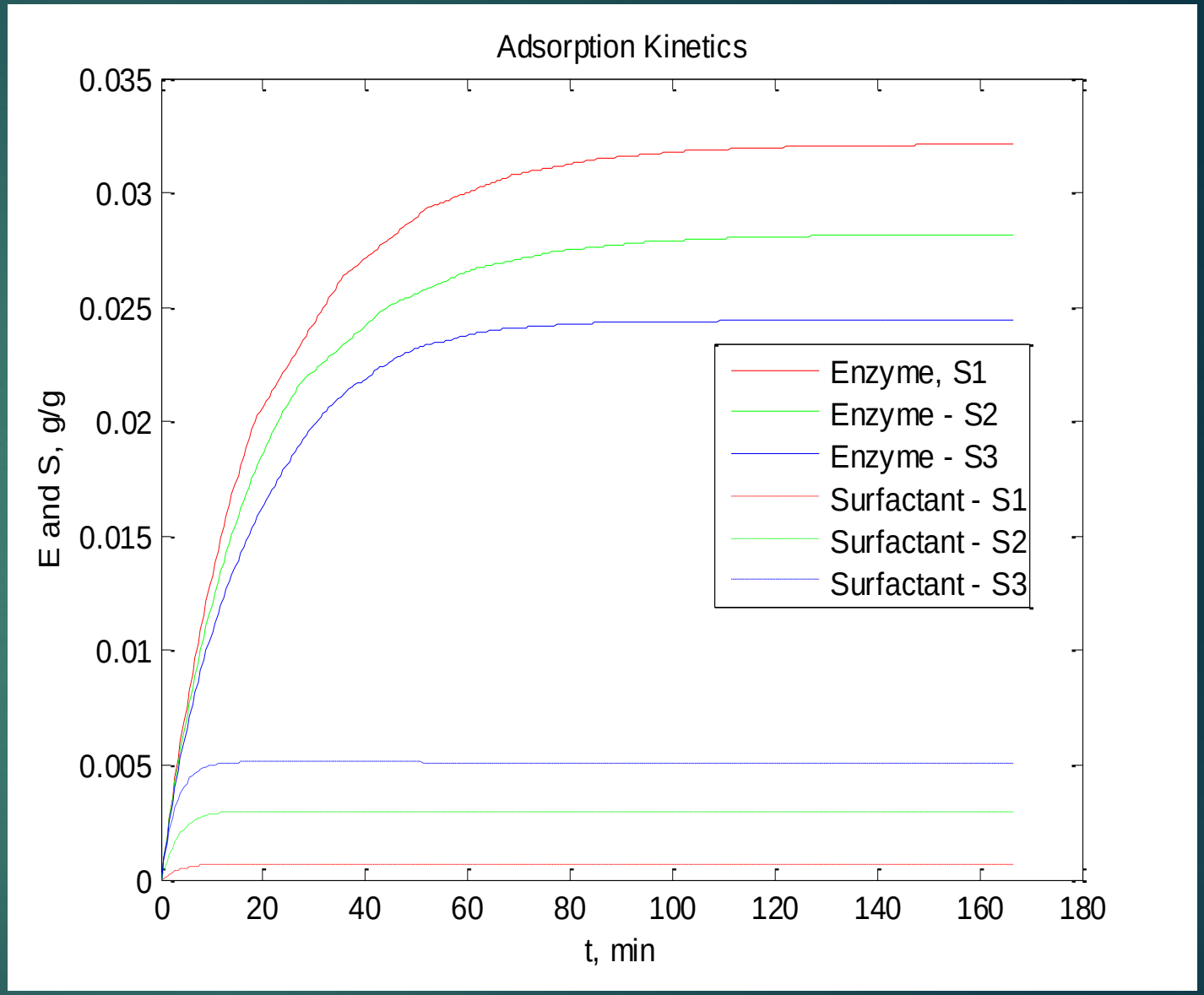
- Fines at 5%
- Ash at 10% of fines
- 50 °C, pH 5,
- Surfactant 3%
- Reaction time 24 hrs

- Model Predicts
  - Sugar concentration
  - Bound-Enzyme concentration
  - Free enzyme concentration
  - Impact of surfactants
  - Optimal parameter windows

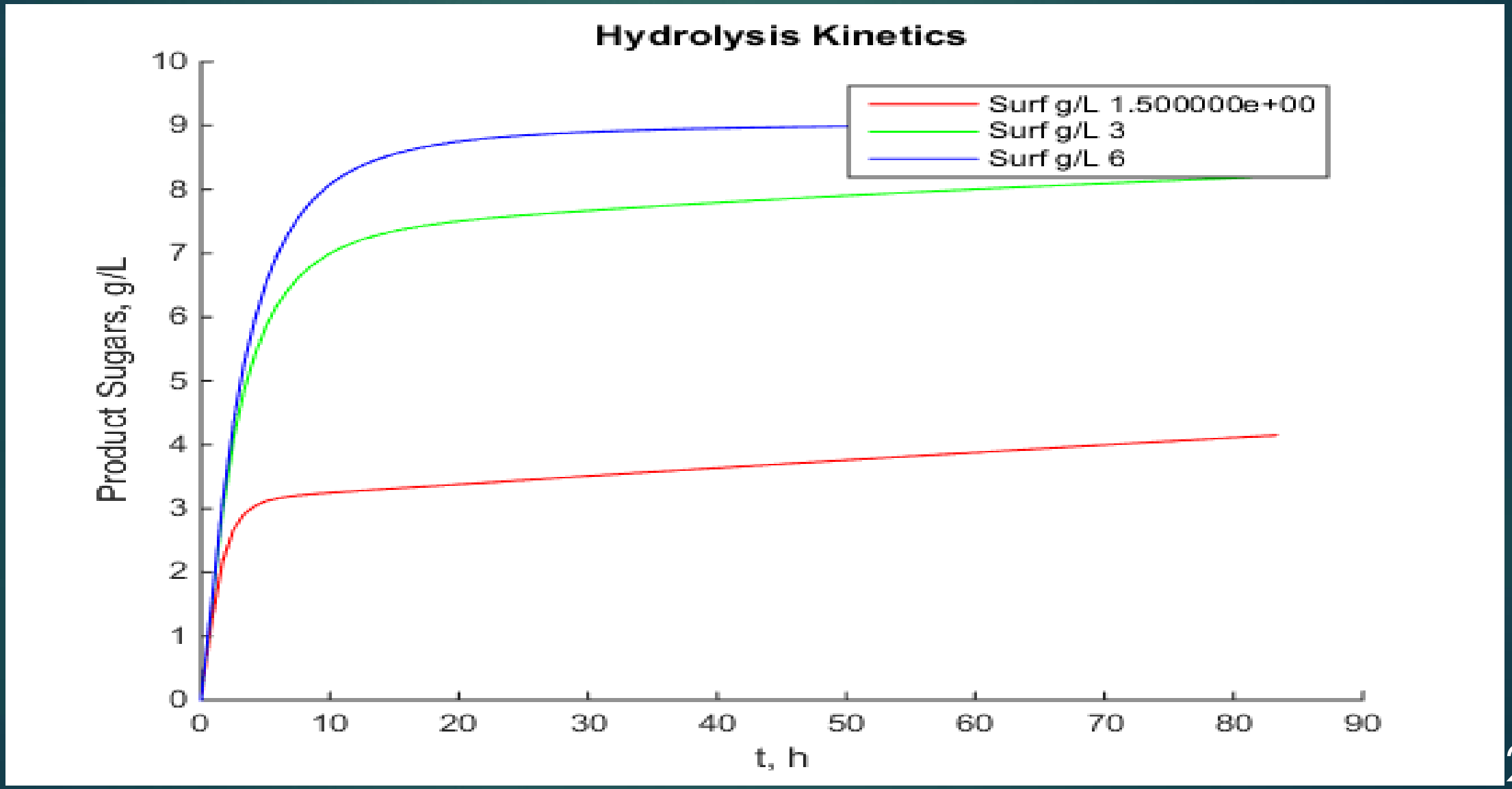
Model is solved by numerical analysis: matlab/minitab

# Enzyme and Surfactant Adsorption on CC.

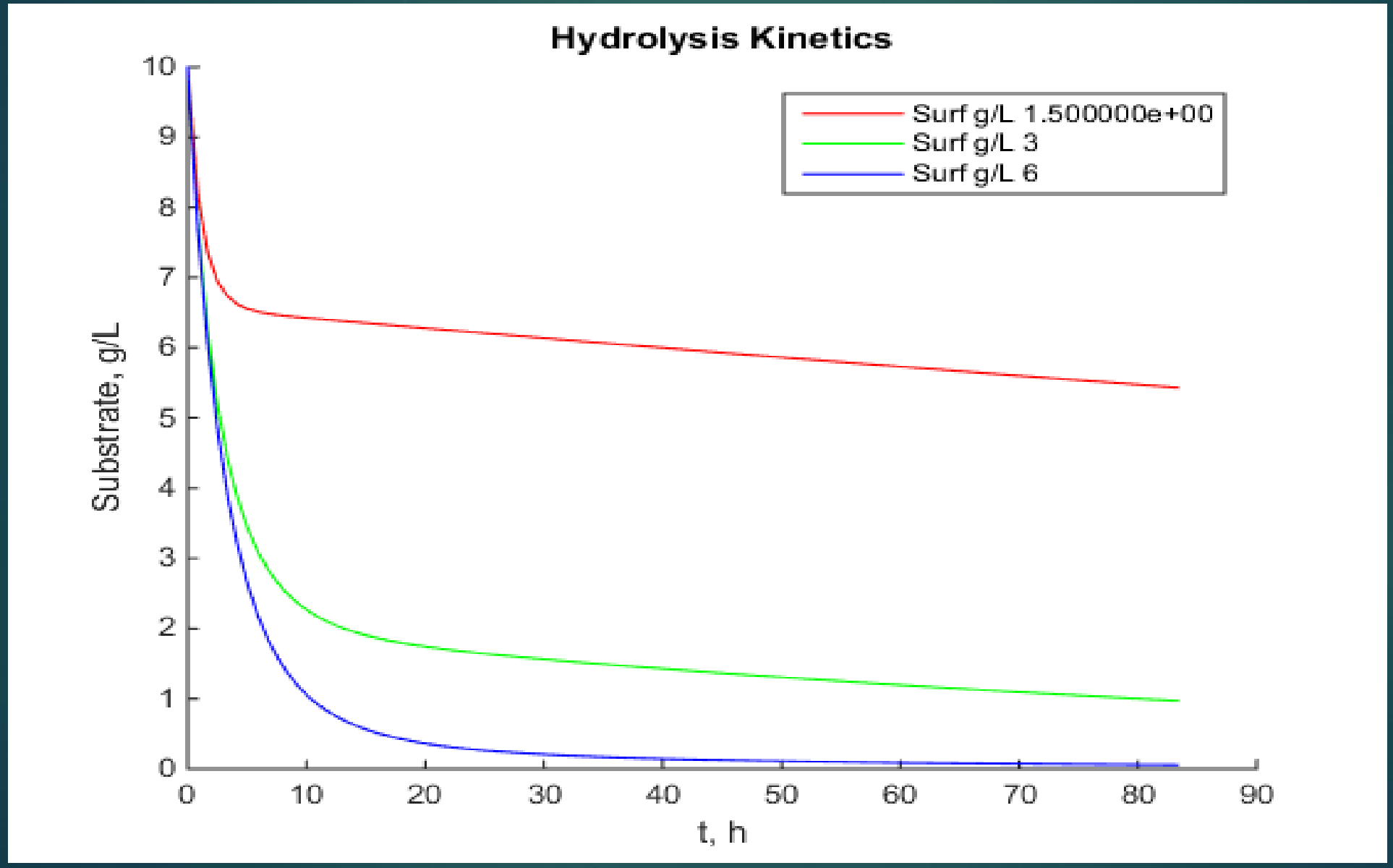
Initially rapid and then saturates (flattening).



# Kinetics of sugar production at Surfactant Dosages of 1.5, 3, and 6 g/L.

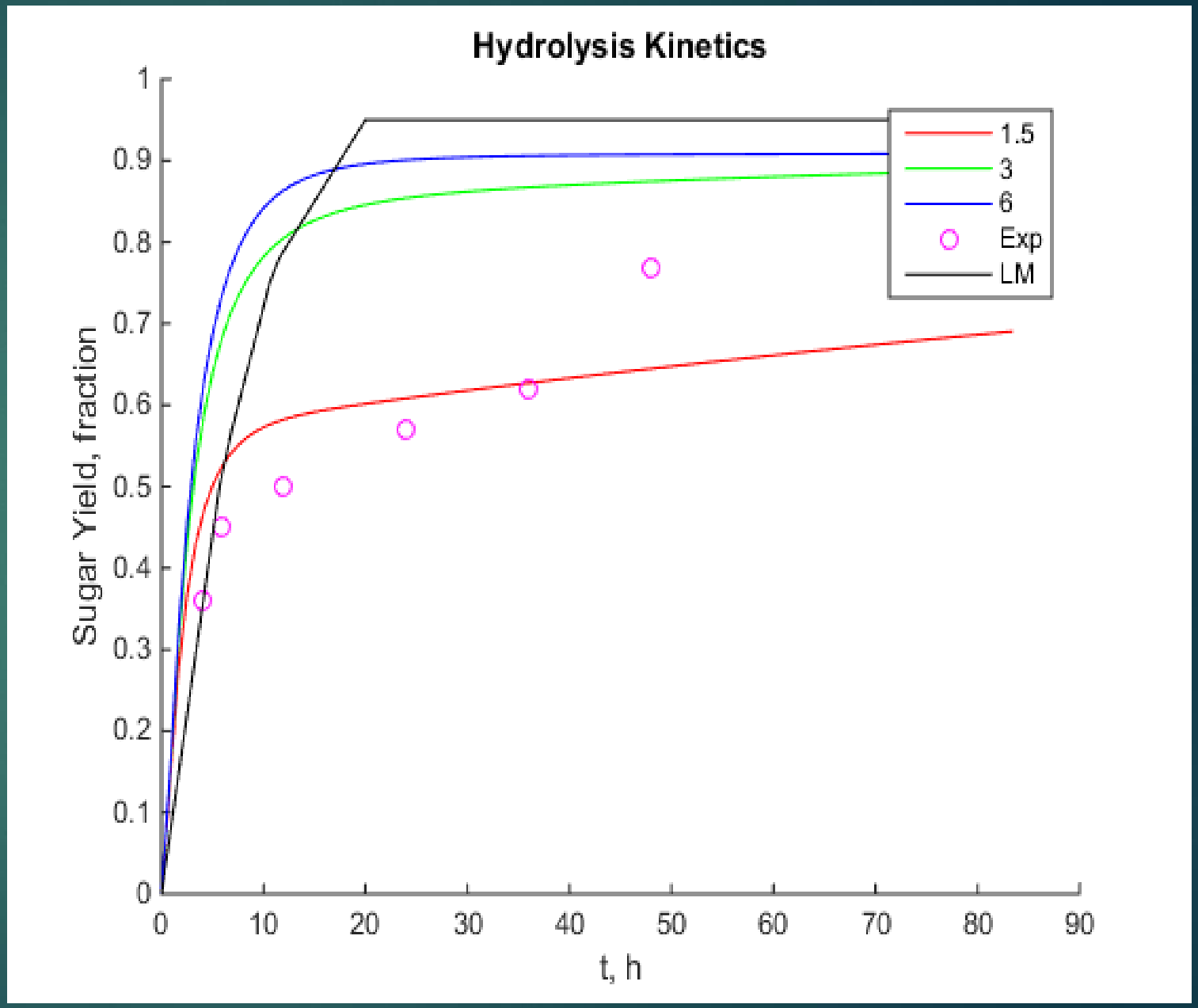


# Kinetics of Fines Dissolution for Surfactant at 1.5, 3, and 6 g/L



Solid Lines - Sugar Yield vs. Time for 3 Surfactant Dosages.

- Min et. al Experimental Data



# Model for Scale Up of Enzyme Hydrolysis

- Feed fibers (fines) and CC particles
- Enzymes adsorb onto the surfaces of fibers and minerals
- Reaction occurs, glucose is released,  enzyme goes back into solution
- Glucose reacts and reduces its activity – inhibition
- Enzyme availability onto CC
- Surfactant adsorps on to CC  leaving the enzymes for glucose formation

# Kinetic Experiments/Statistical Modeling/Response Square Model (RSM) for Sugar Yield

$$Sugar\ Yield, s = A_0 + A_1 c_f + A_2 d_f + A_3 c_E + A_4 c_S + A_5 t + A_{12} c_f d_f + A_{13} c_f c_E + A_{14} c_f c_S + A_{15} c_f t + A_{23} c_E d_f + A_{24} d_f c_S + A_{25} d_f t + A_{34} c_E c_S + A_{35} c_E t + A_{45} c_s t + A_{11} c_f^2 + A_{22} d_f^2 + A_{33} c_E^2 + A_{44} c_S^2 + A_{55} t^2$$

*where,*

$s$ = *Yield in g/L (concentration)*

$c_E$ = *Enzyme dosage (FPU/g)*

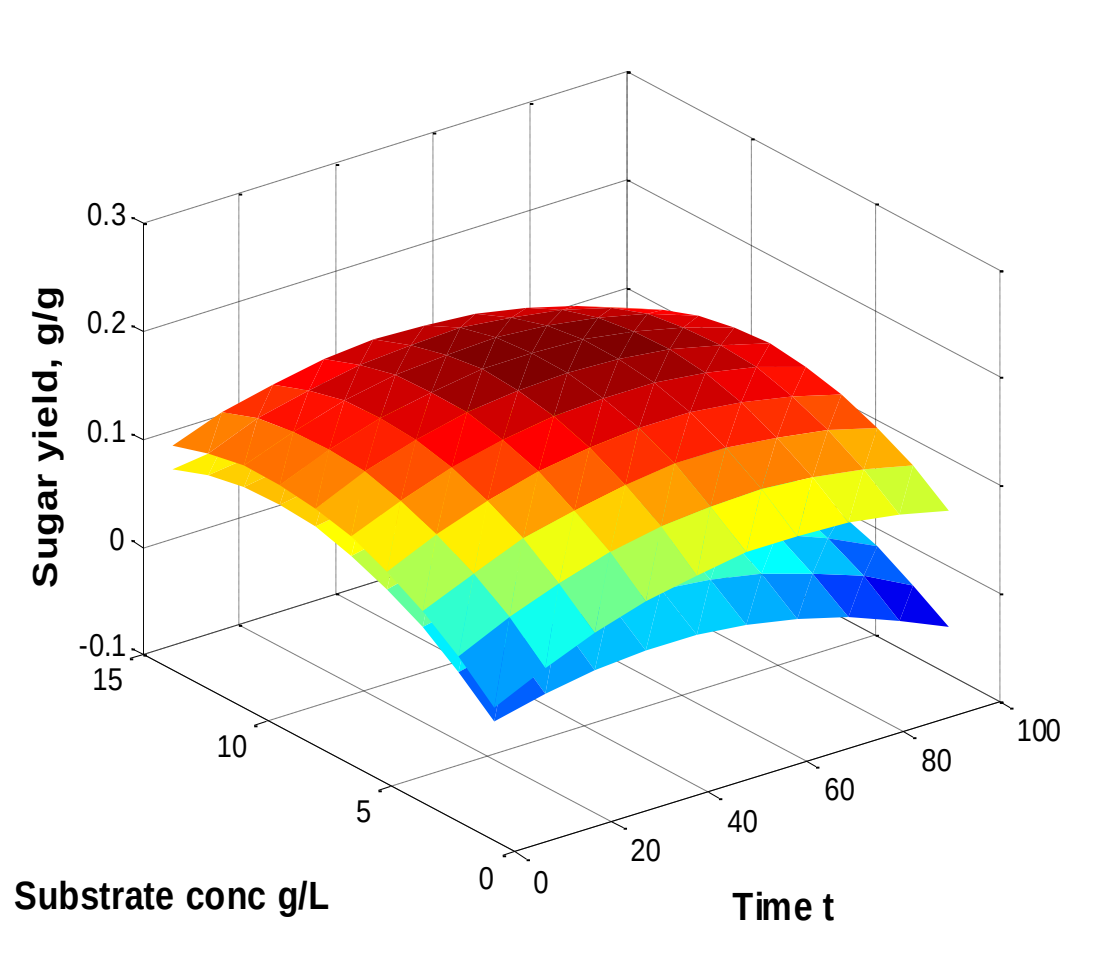
$c_S$ = *Surfactant dosage (g/g, dry fines)*

$c_f$ = *Fines concentration (g/g)*

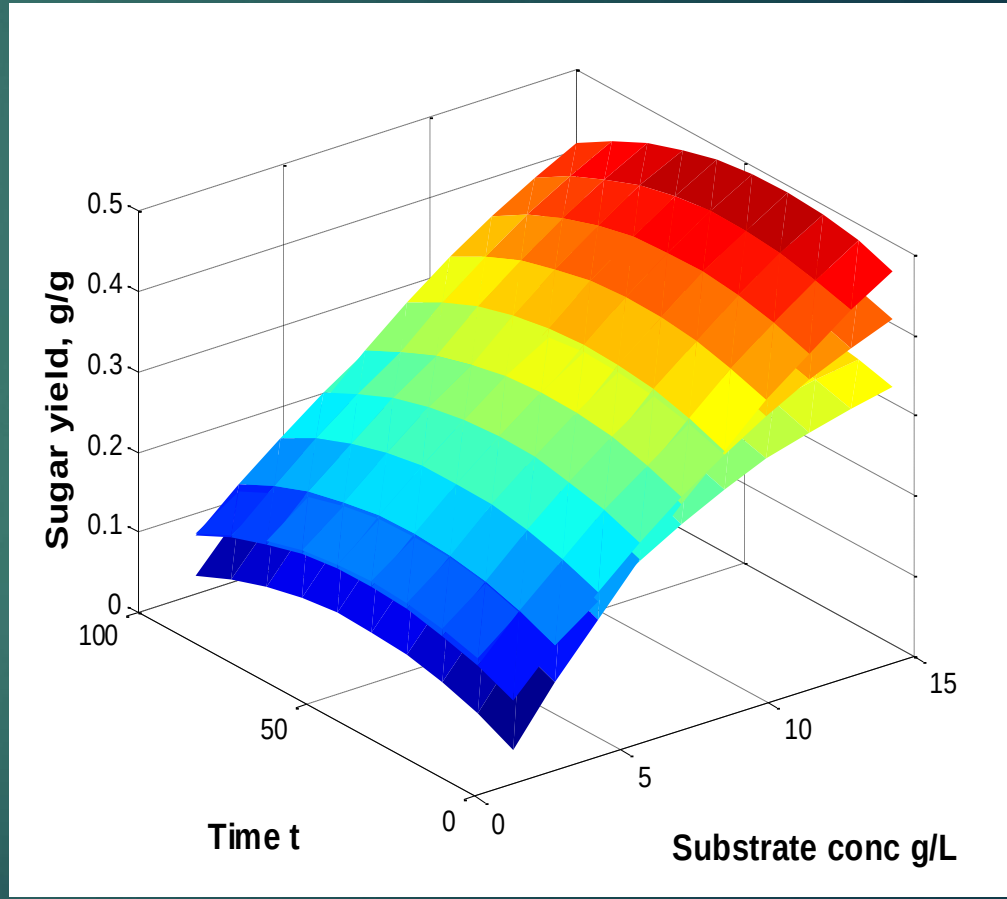
$d_f$ = *particle size (µm)*

$t$ = *Retention time (hrs)*

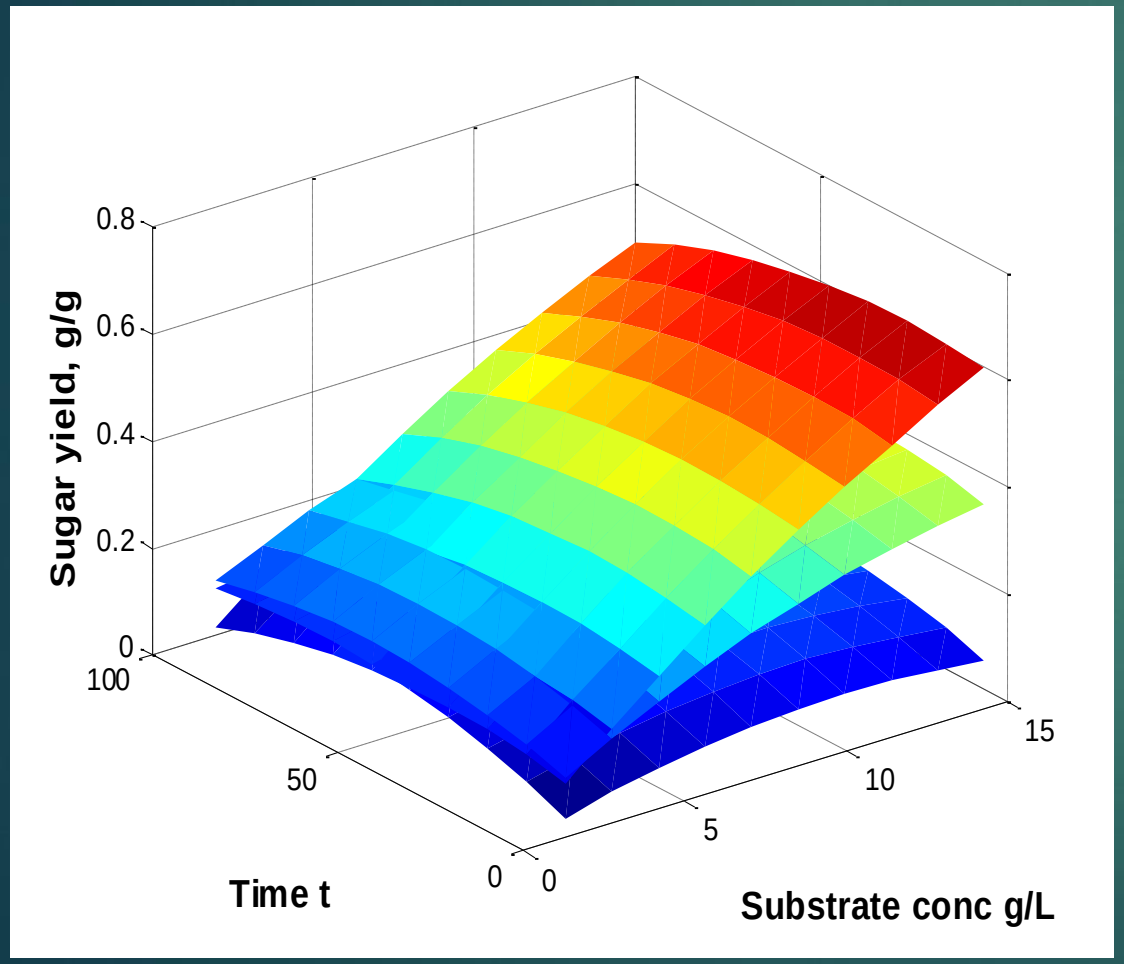
Prediction of Sugar Yield with Time and Substrate Concentrations. Enzyme at 2 and 5 FPU/g



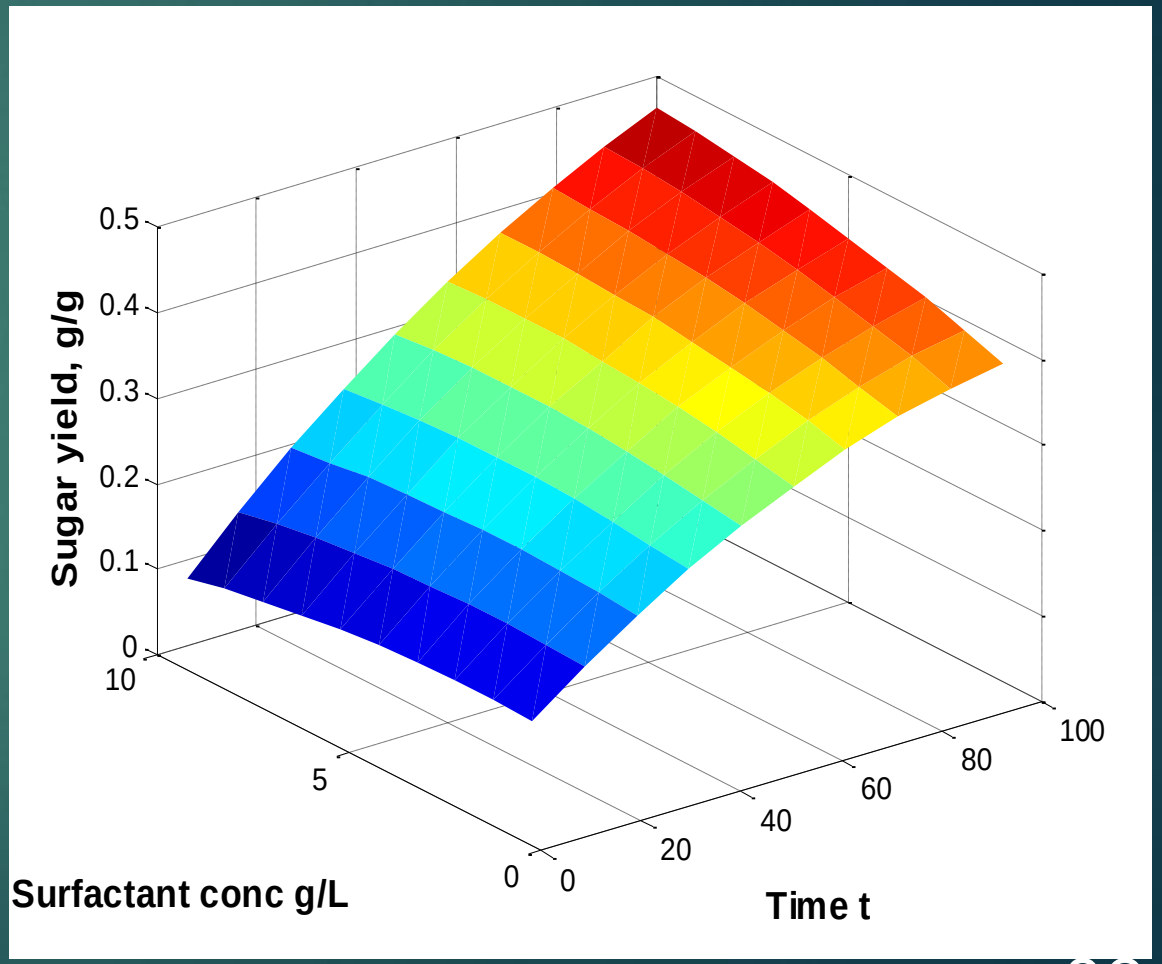

Prediction of Sugar Yield with Time and Substrate Concentrations. Surfactant Concentrations 3, 6 and 9%.



Prediction of Sugar Yield with Time and Substrate Concentrations. Enzyme at 2, 10 and 20 FPU/g.




Prediction of Sugar Yield with Time and Surfactant Concentrations.



# Kinetic Model Conclusions

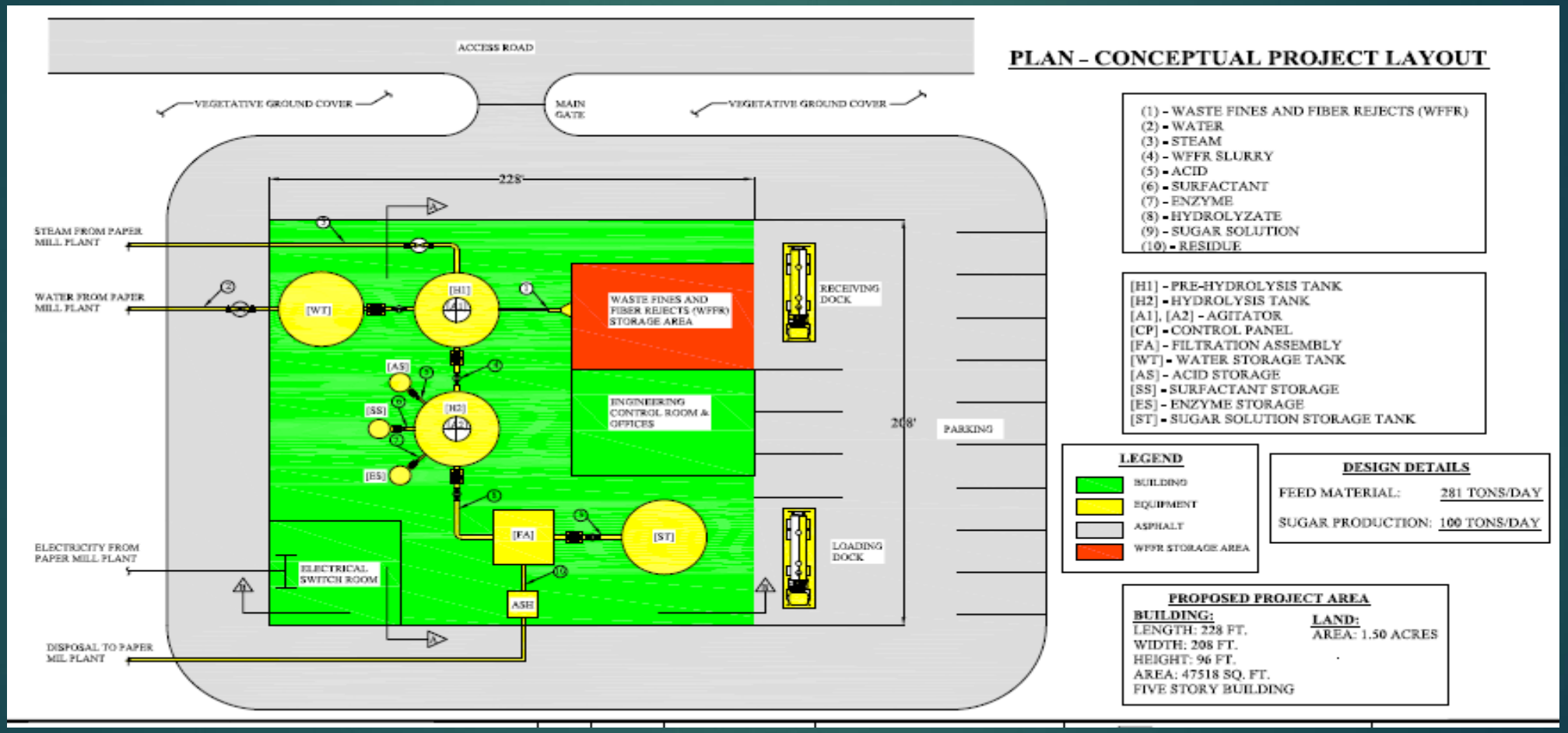
- Reaction kinetics were established for scale up. Impact of filler/inhibitor on enzymes & surfactants are quantified
- Reaction rates for best product yield quantified
- CC slows down the reaction & limits sugar yield
- Surfactants allow for faster hydrolysis (enzymatic activity) & higher yield
- Model can now be used for scale up material balance

# Process Design, Optimization & Economic Analysis

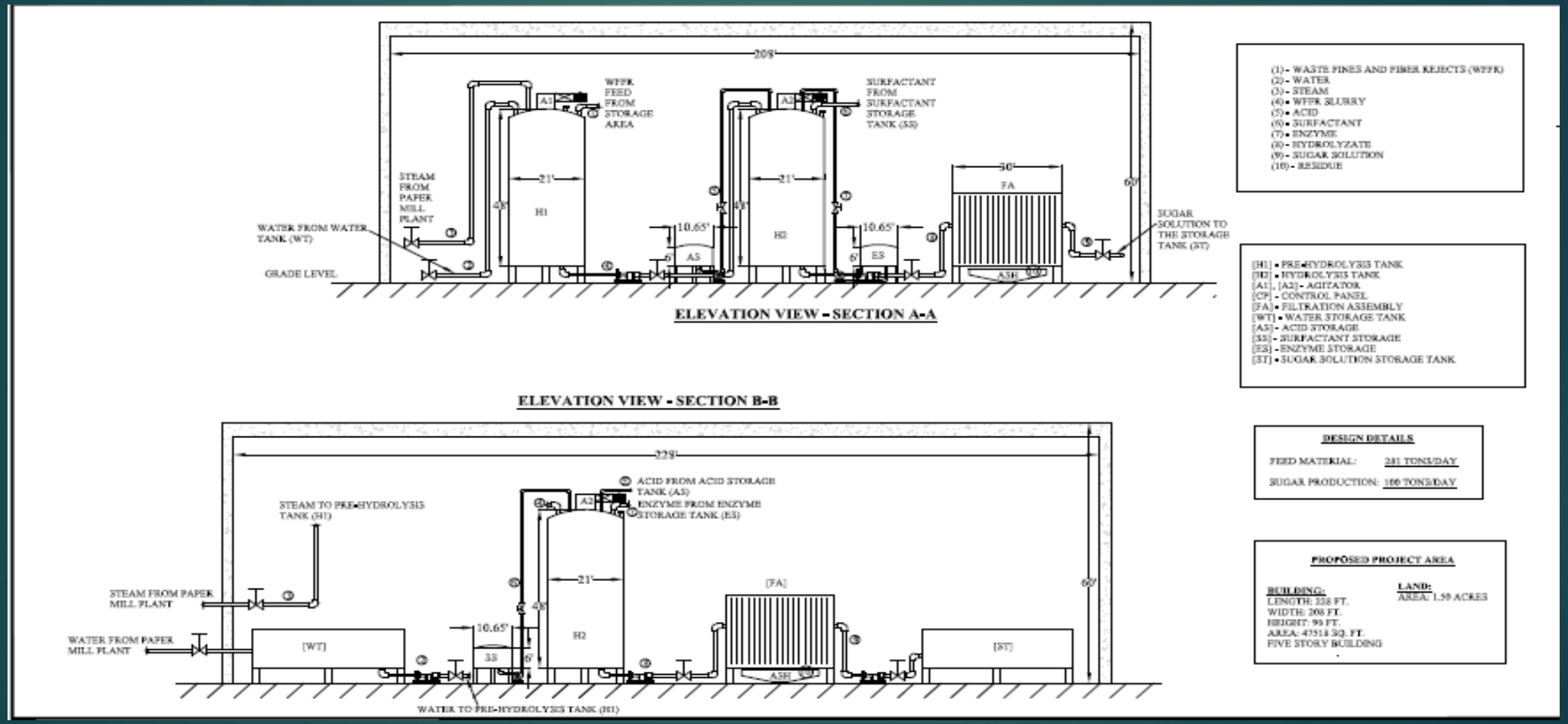
Established reaction kinetics, cellulose chemistry based Response Square Model (RSM) formula for sugar yield was utilized to develop the following :

- Material Balance
- Economic evaluation parameters
- Graphical Analysis – Guidance for Process Development
- Cost Benefit Analysis

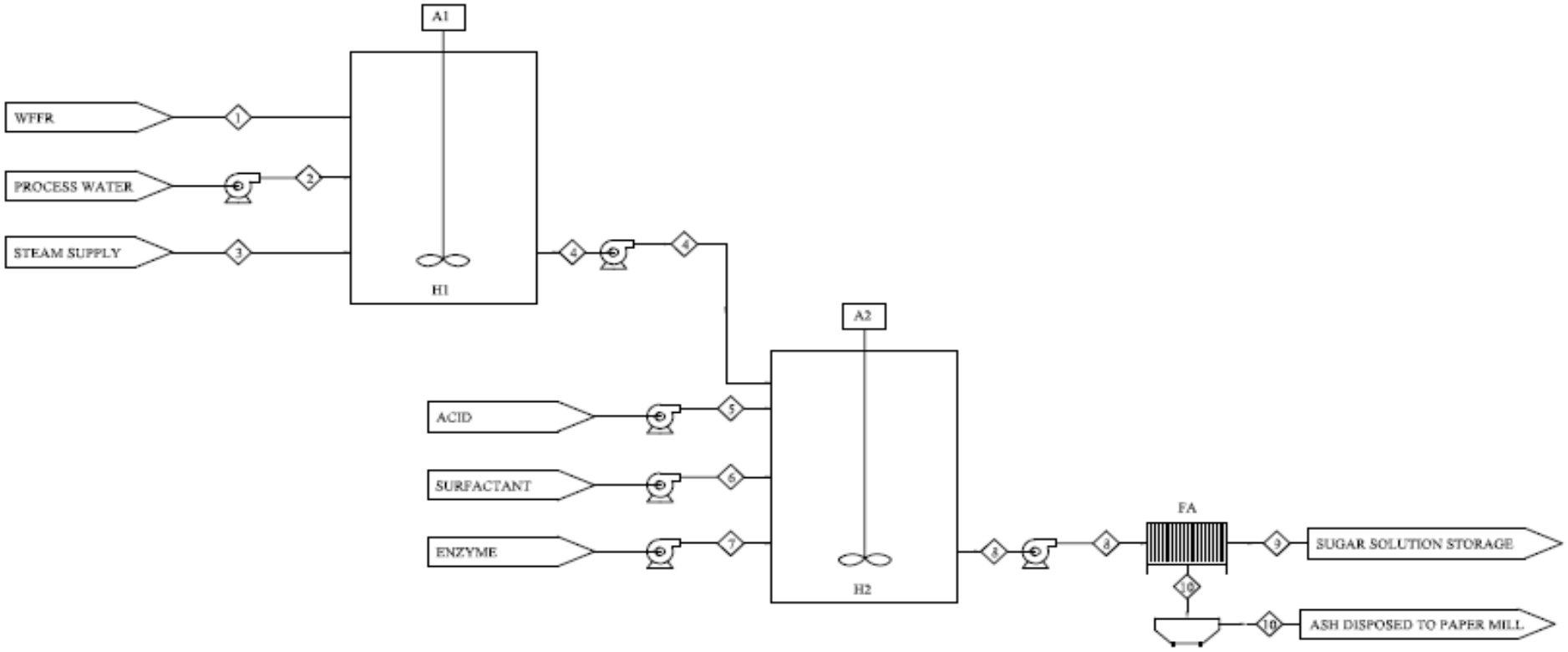
# Conceptual Process Development Plan for Hydrolysis Plant – 100 kg/day Basis



# Conceptual Process Development Plan – Elevation View



# Conceptual Process Development Plan – Process Schedule




| Process Description | WFFR | Water Flow | Steam | Slurry | Acid | Surfactant | Enzyme | Hydrolysate | Sugar Solution | Ash |
|---|---|---|---|---|---|---|---|---|---|---|
| Node | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Total Flow, lbs./hr | 23427 | 190774 | 180 | 214201 | 3444 | 0 | 1230 | 218875 | 204819 | 14056 |

# Input Parameters for Material Balance – Spread Sheet

- $Total\ Water\ Required\ in\ the\ Mixture, W\ (l) = \frac{100}{C_f(\%)} \times \frac{WFFR\ (kg)}{Density\ of\ Water{=}1\ kg/l}$
- $Water\ to\ be\ Added, W_a\ = W - \frac{WFFR\ (kg)}{Water\ Density\ \left(=1\frac{kg}{l}\right)} \times \frac{W_w(\%)}{100}$
- $E = \ WFFR\ (kg) \times \frac{E_D\left(\frac{FPU}{g}\right)}{E_A\left(\frac{FPU}{ml}\right)} \times \frac{1000\frac{g}{kg}}{1000\frac{ml}{l}}$
- $S = WFFR\ (kg) \times \frac{S_s(\%)}{100} \times \frac{1}{Water\ Density\ \left(=1\frac{kg}{l}\right)}$
- $A = \ W\ \times pHR\ (= 0.004)$
- $T_L = W + E + S + A$

# Material Balance View

## Input

| | | |
|---|---|---|
| Consistency | 8 | % |
| Fines and Fibre rejec | 6327948 | kg |
| cellulose (@40% WF | 2531179 | Kg |
| Ash (@40% WFFR) | 2531179 | Kg |
| Others (@20% WFFR | 1265590 | |
| Moisture | 50 | % |
| Water | 36385704 | L |
| Water from fines | 3163974 | L |
| Total water | 39549678 | L |
| pH Maintenance Rat | 0.014 | L |
| Acid(@14 mL per {lit | 442956.39 | L |
| % Surfactant | 3 | % |
| Surfactant (@3% bas | 94919.23 | L |
| Enzyme activity | 200 | FPU/mL |
| Enzyme Dosage | 9 | FPU |
| Enzyme | 284758 | L |
| Total Volume of liqu | 40372311 | |

linked to Eco Model, Empirical Formula

## Hydrolysis Reactor

| | | |
|---|---|---|
| Time | 24 | h |
| Concentra | 31.60582 | g/L |
| Yield base | 20.2 | % |
| Yield base | 50.4 | % |
| Sugar | 1250000 | Kg |

## Filtration

| | | |
|---|---|---|
| Efficiency | 80 | % |
| Sugar (P, kg/day) | 1000000.0 | Kg |
| Volume of Filtrate | 32297849 | L |
| Residue | 5327948 | |
| Volume of water in Residue | 8074462 | |

# Economic Evaluation

- $\$_{Enz} = \$_{EU} \times \mathrm{E}\,(L)$
- $\$_{Surf} = \$_{SU} \times \mathrm{S}\,(L)$
- $\$_{Acid} = \$_{AU} \times A\,(L)$

where,

$\$_{Enz}$ = total cost of enzyme for the batch reaction/run

$\$_{EU}$ = unit cost of enzyme per liter of enzyme solution

$\$_{Surf}$ = total cost of surfactant for the batch reaction/run

$\$_{SU}$ = unit cost of surfactant per liter

$\$_{Acid}$ = total cost of acid for the batch reaction/run

$\$_{AU}$ = unit cost of acid per liter

# Cost ($) Items

- Operating Costs ($):
  - WFFR, Water, Steam, Acid, Surfactant, Enzyme
  - Electrical - Pumps', stirrer, filter, cleaning
  - General operation & maintenance
- Installation Costs ($)
  - Hydrolysis tanks, agitator, stirrer, pumps, control panel, filter assembly, feed tanks, metered pumps, water tank, storage tanks, connecting pipes, heating elements, installation & labor

# Best Economical Case

- Sugar concentration : 27 g/L
- Enzyme dosage : 1.5 FPU/g
- Surfactant concentration : 1%
- Fines consistency : 7%
- Reaction time : 36 hrs
- Reaction temperature : 50 °C

NOTE: Does NOT correspond to best reaction kinetics dataset

# Summary of Best Case Scenarios for Scale Up

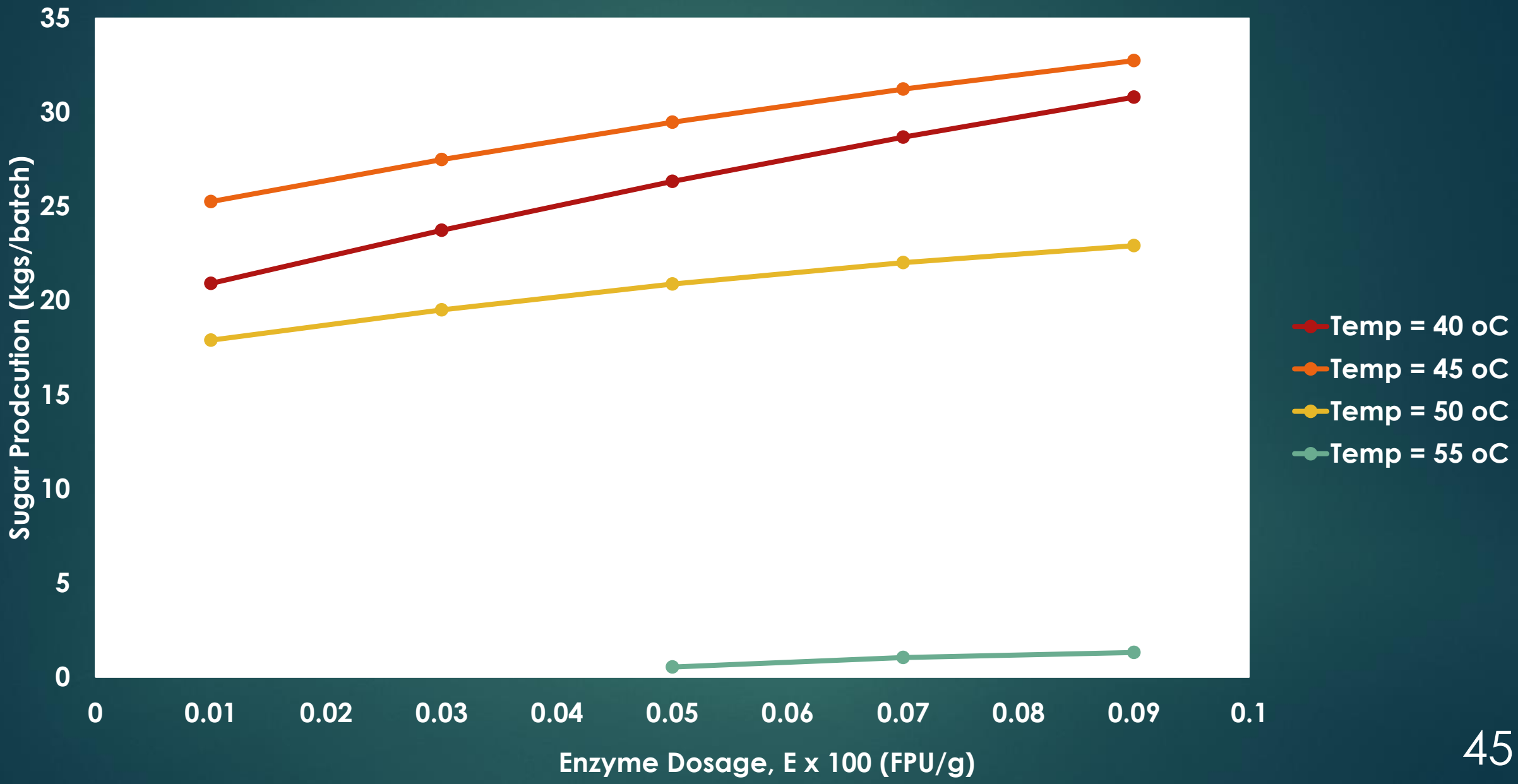
| | Waste Throughput (kg/batch) | Fines Consistency (%) | Enzyme Dosage (FPU/g) | Temperature Range (°C) | Reaction Time (hrs) | Surfactant (%) | Max. Sugar Yield (kg) | Yield (kg Sugar/ kg WFFR) | Figure Reference |
|---|---|---|---|---|---|---|---|---|---|
| **1.** | 100 kg | 7% | 9 | 45 °C | 12 | 1 | 33 | 0.33 | Figure 4.12 |
| **2.** | 100 kg | 7% | 5 | 45 °C | 24 | 1 | 40 | 0.40 | Figure 4.13 |
| **3.** | 100 kg | 7% | 9 | 45 °C | 12 | 1 | NA | 0.33 | Figure 4.14 |
| **4.** | 100 kg | 7% | 5 | 45 °C | 24 | 1 | NA | 0.40 | Figure 4.15 |
| **5.** | 300,000 kg | 7% | 9 | 45 °C | 12 | 3 | 100,000 | 0.33 | Figure 4.16 |
| **6.** | 300,000 kg | 7% | 9 | 40 °C | 24 | 3 | 120,000 | 0.40 | Figure 4.17 |
| **7.** | 300,000 kg | 7% | 9 | 45 °C | 12 | 3 | 100,000 | 0.33 | Figure 4.18 |
| **8.** | 300,000 kg | 7% | 9 | 40 °C | 24 | 3 | 120,000 | 0.40 | Figure 4.19 |
| **9.** | 300,000 kg | 11% | 9 | 40 °C | 12 | 3 | 100,000 | 0.33 | Figure 4.20 |
| **10.** | 300,000 kg | 11% | 9 | 40 °C | 24 | 3 | 110,000 | 0.36 | Figure 4.21 |
| **11.** | 300,000 kg | 7% | 1 | 50 °C | 36 | 1 | 120,000 | 0.36 | See Note |

Note: Case #11 best case: 1 - 10 are based on reaction kinetics

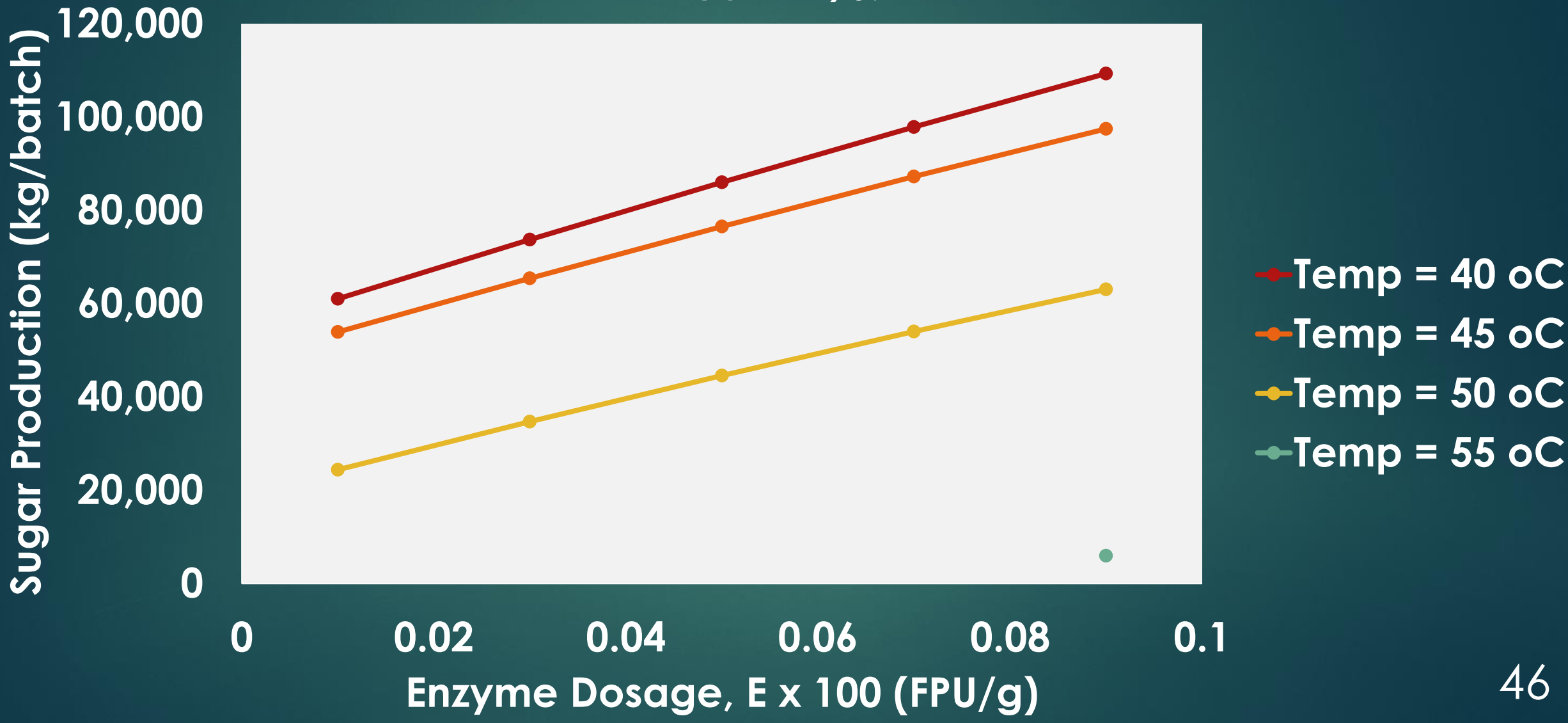
# Economic Evaluation - Spreadsheet

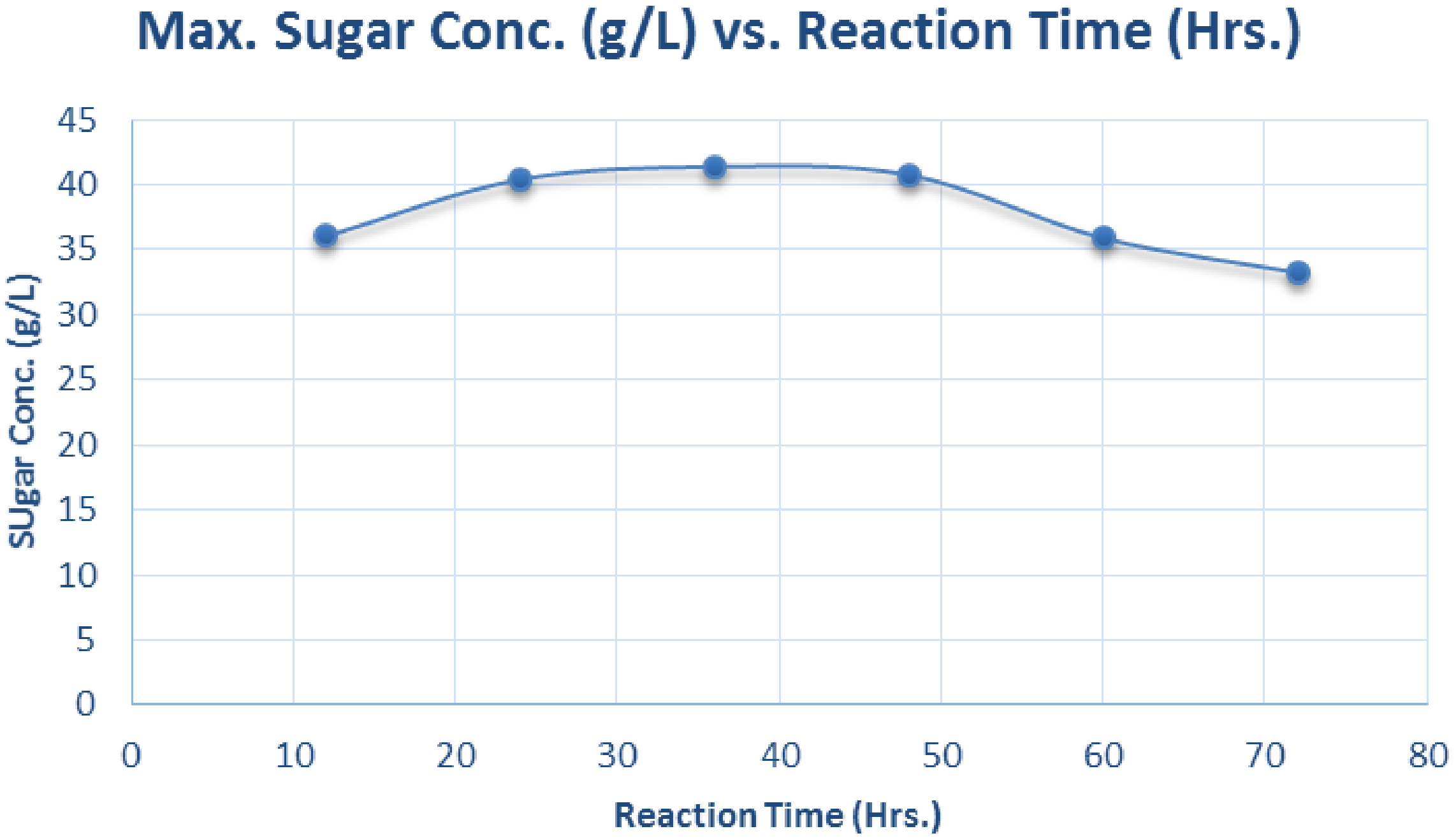
| Operating Cost Model Inputs | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Input Variables | | Per Unit $ | Process Operating Variables | Kg or Hrs | $ | | |
| Concentration (C, g/L) | 20 | | $ - | WFFR (kgs) | 300000 | $ - | | |
| Production (P, kg/day) | 61,620.00 | | $ - | Water (w, kgs) | 2850000 | $ - | | |
| Tank size factor | 1.5 | | $ 0.05 | Steam (St, kgs) | | $ - | | |
| Pre-Hydrolysis Tank Vol (L) | 4275000 | | $ 10.00 | Acid (Ac, kgs) | 42000 | $ 420,000 | | |
| Hydrolysis Tank Vol (L) | 4275000 | | $ 20.00 | Surfactant (SS, kgs) | 9000 | $ 38 | | |
| Tank Diameter (m) | 1.21 | | $ 20.00 | Enzyme (E, kgs) | 30000 | $ 600,000 | | |
| Stirrer Size (dia, m) | 0.36 | | $ 10.00 | Operator Cost (hrs, $) | 24 | $ 240.00 | | |
| Operator Cost ($/hr) | 15 | | $ 1.00 | Pumps Op. Costs | 24 | $ 0.59 | | |
| Reaction Time (hrs) | 24 | | $ 1.00 | Heating Cost ($/tank vol.) | 24 | $ 24.00 | | |
| Filter Size (sq. ft.) | 64 | | $ 1.00 | Stirring Cost ($/reac time) | 24 | $ 24.00 | | |
| Filter Factor (sq. ft/kg) | 1 | | $ 0.01 | Filter Cleaning ($) | 2464800 | $ 12,324.00 | | |
| Hydrolysis Tank Ht. (h, m) | 3718 | | | | | | | |
| **MODEL OUTPUT** | | | | | | | | |
| **Total Install Cost ($)** | **$ 3,480** | | | | | | | |
| **Total Operating Cost ($/batch op.)** | **$ 1,032,651** | | | | | | | |
| **Cost per kg of Sugar** | **$ 16.81** | | | | | | | |
| | | | | | | | | |
| Electric/Unit (kWh) Cost ($ | $ 0.26 | | | | | | | |
| | | | | **Total Operating Cost ($/batch op.)** | | **$ 1,032,651** | | |

| Plant Installation Cost | QUANTITY | cost per item ($) | Cost ($) |
|---|---|---|---|
| Hydrtolysis Tank | 1 | $ 5,365,960.73 | $ 1,136 |
| Pre-Hydrolysis Tank | 1 | $ 5,365,961 | $ 1,136 |
| Agitator Bridge | 2 | $ 4,200 | $ 2 |
| Sitrrer Cost | 2 | $ 3,725 | $ 2 |
| Water Pump | 3 | $ 1,160 | $ 1 |
| Metered Pumps | 3 | $ 466 | $ 0 |
| Control Panel | 1 | $ 2,000 | $ 0 |
| Filter Assembly | 64 | $ 200 | $ 3 |
| Acid Tank/Storage | 1 | $ 1,500 | $ 0 |
| Surfactant Storage | 1 | $ 79 | $ 0 |
| Enzyme Storage | 1 | $ 148 | $ 0 |
| Pure Water Storage Tan | 1 | $ 1,074 | $ 0 |
| Product Storage Tank | 1 | $ 1,074 | $ 0 |
| Waste Storage | 1 | $ 797 | $ 0 |
| Connecting pipes | 20 | $ 100 | $ 0 |
| Heating Element Cost | 14132 | 20 | $ 60 |
| | | | $ - |
| Miscellaneous | | | $ - |
| Installation & Labor | (Assume: | | $ 1,140 |
| | | **Total Install Cost ($)** | **$ 3,480** |

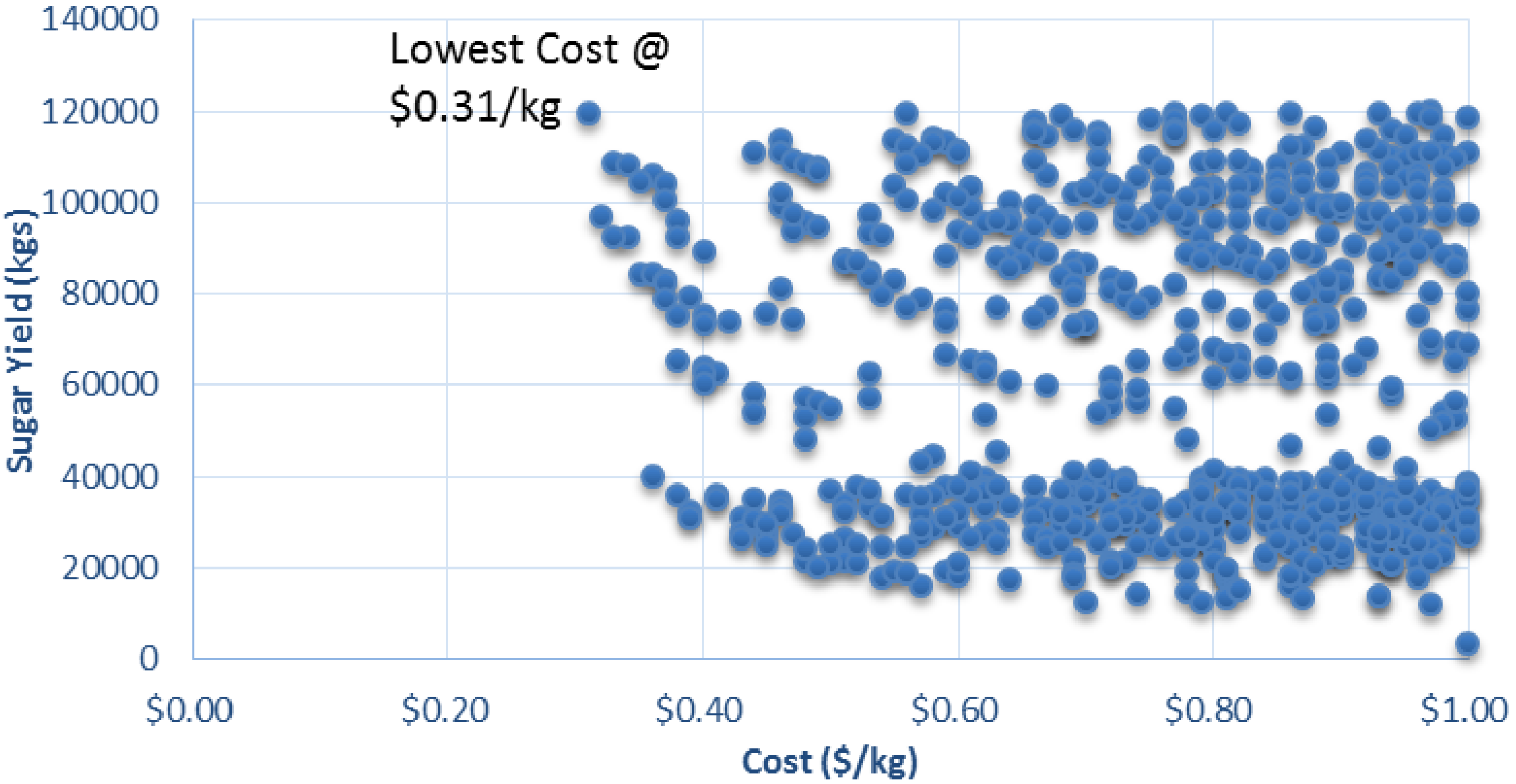

WFFR = 100 kgs, Time = 12 hrs, Surf. = 1%, Fines=7%
Sugar Prodcution (kgs/batch)
35
30
25
20
15
10
5
0
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.1
Enzyme Dosage, E x 100 (FPU/g)
Temp = 40 oC
Temp = 45 oC
Temp = 50 oC
Temp = 55 oC

# WFFR = 300,000 kgs, Time = 24 hrs, Surf. = 3%, Fines=11%.

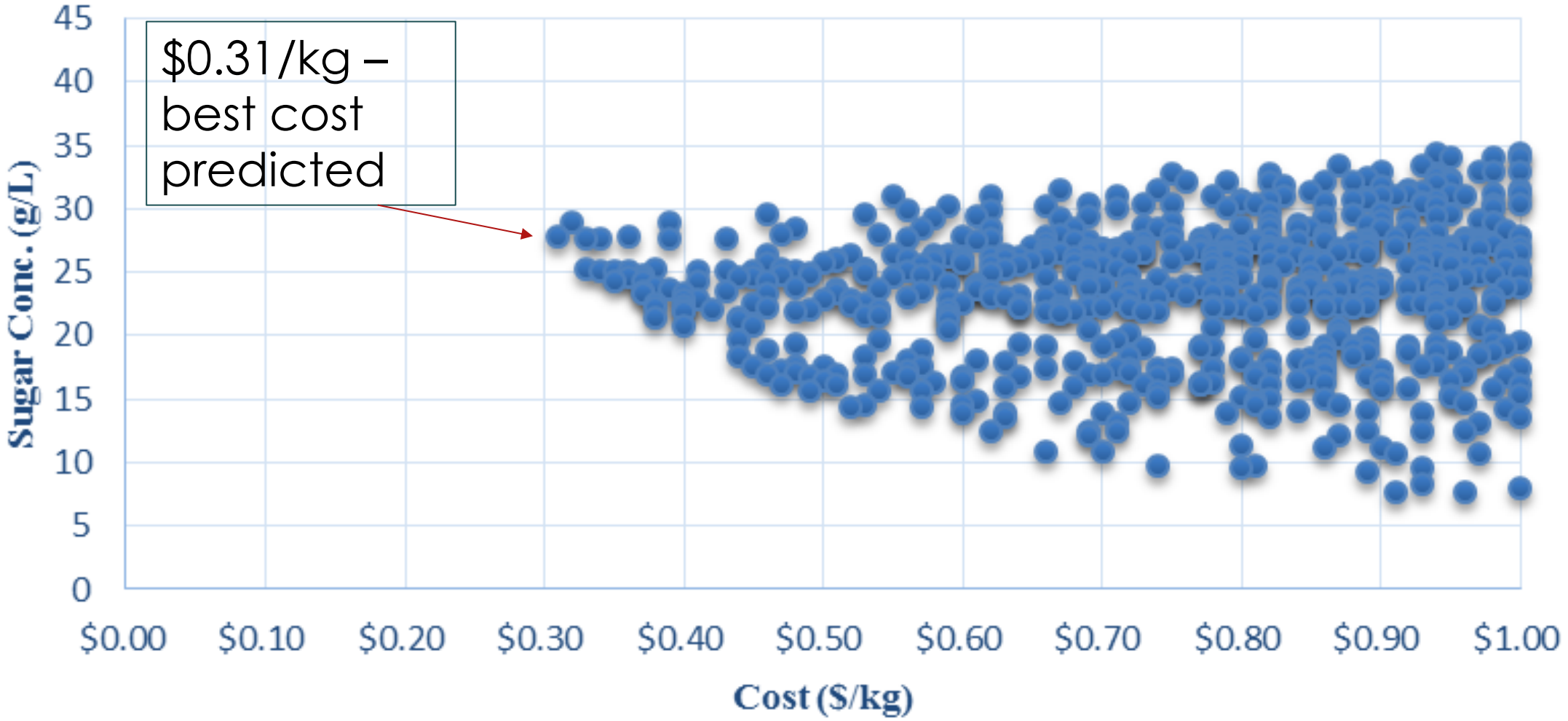



Max. Sugar Conc. (g/L) vs. Reaction Time (Hrs.)
SUgar Conc. (g/L)
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
Reaction Time (Hrs.)

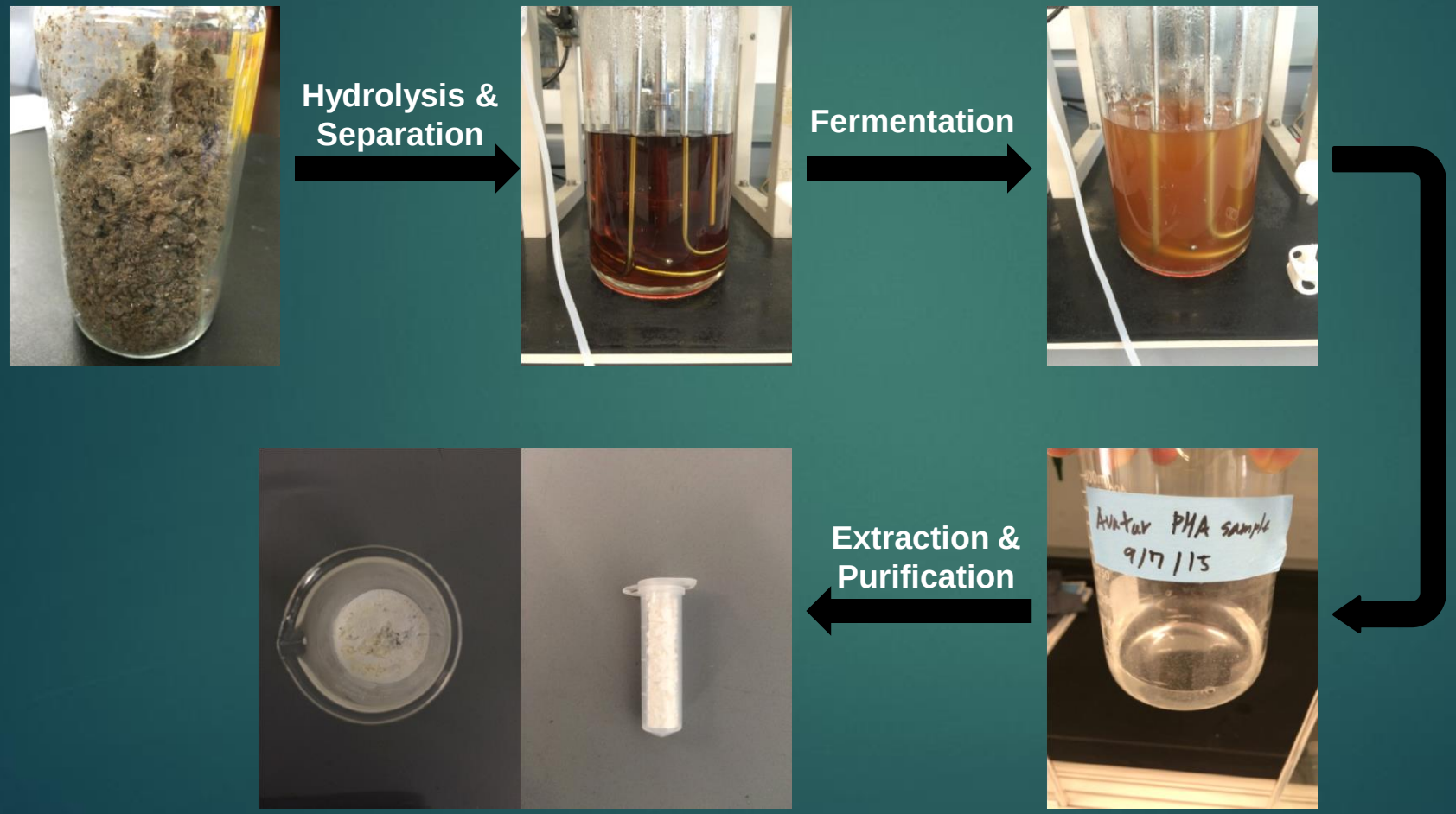
# Sugar Yield (kgs) vs. Cost ($/kg) < $1



# Sugar Conc. (g/l) vs. Cost ($/kg) < $1



# We have a Bio Plastic  Solutions in Scale-Up Level!



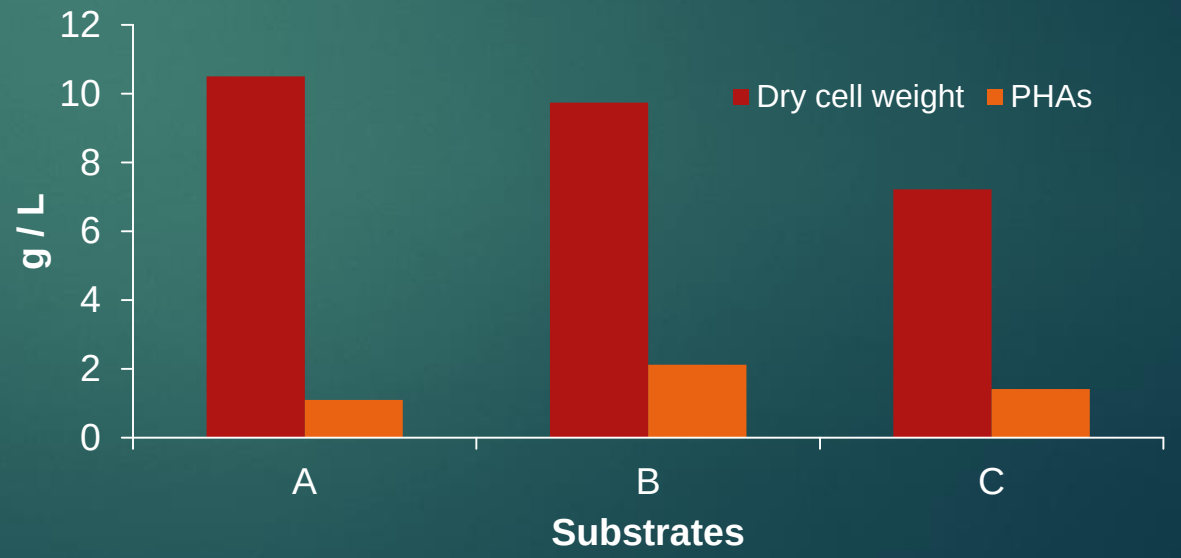
# PHAs Production

| Substrate | DCW[1] (g/L) | PHA (g/L) | Yield on DCW | DCW (%, g/g of sugars) | PHA (%, g/g of sugars) |
|---|---|---|---|---|---|
| A | 10.5 | 1.1 | 0.11 | 53 | 6-7 |
| B | 9.74 | 2.12 | 0.22 | 49 | 11-13 |
| C | 7.22 | 1.41 | 0.2 | 36 | 7-9 |

1. DCW: Dry Cell Weight

Various kinds of paper mill waste are available for substrate.

The yields of PHAs is lower than references showing 20-30% PHA yields maximum. The difference may because no aeration and pH control were used. Shake Flask experiments.



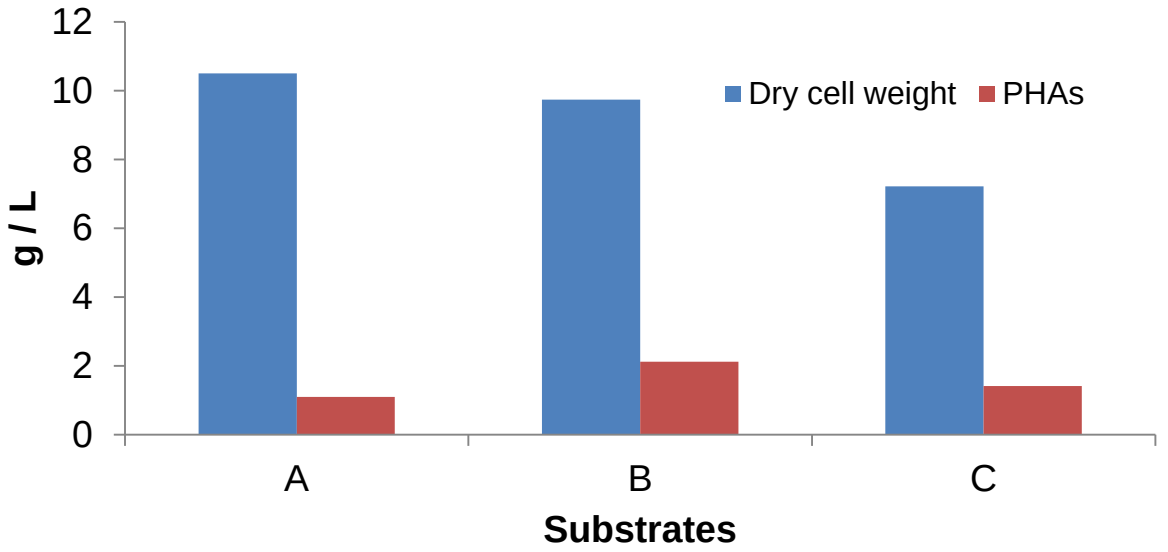
# PHAs Production

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**A 1.5-ml microcentrifuge tube containing P(3HB) produced from hydrolyzate batches (10% consistency) fermented by *B. cepacia***



# THANK YOU