Bioprocess intensification: Challenges related to transfer limitation

2nd workshop at CRDB/SBFT-HUST (Hanoi, VN)

27-30th June 2016

INVESTIGATION OF PHYSICAL MECHANISMS DURING DECONSTRUCTION OF PRETREATED LIGNOCELLULOSIC MATRIXES: FROM PURE ENZYMATIC ACTIVITY TO COCKTAIL

TUAN LE

PHD STUDENT

LISBP LABORATOIRE D'INGÉNIERIE DES SYSTÈMES BIOLOGIQUES ET DES PROCÉDÉS

INRA SCIENCE & IMPACT

cnrs

INSA TOULOUSE

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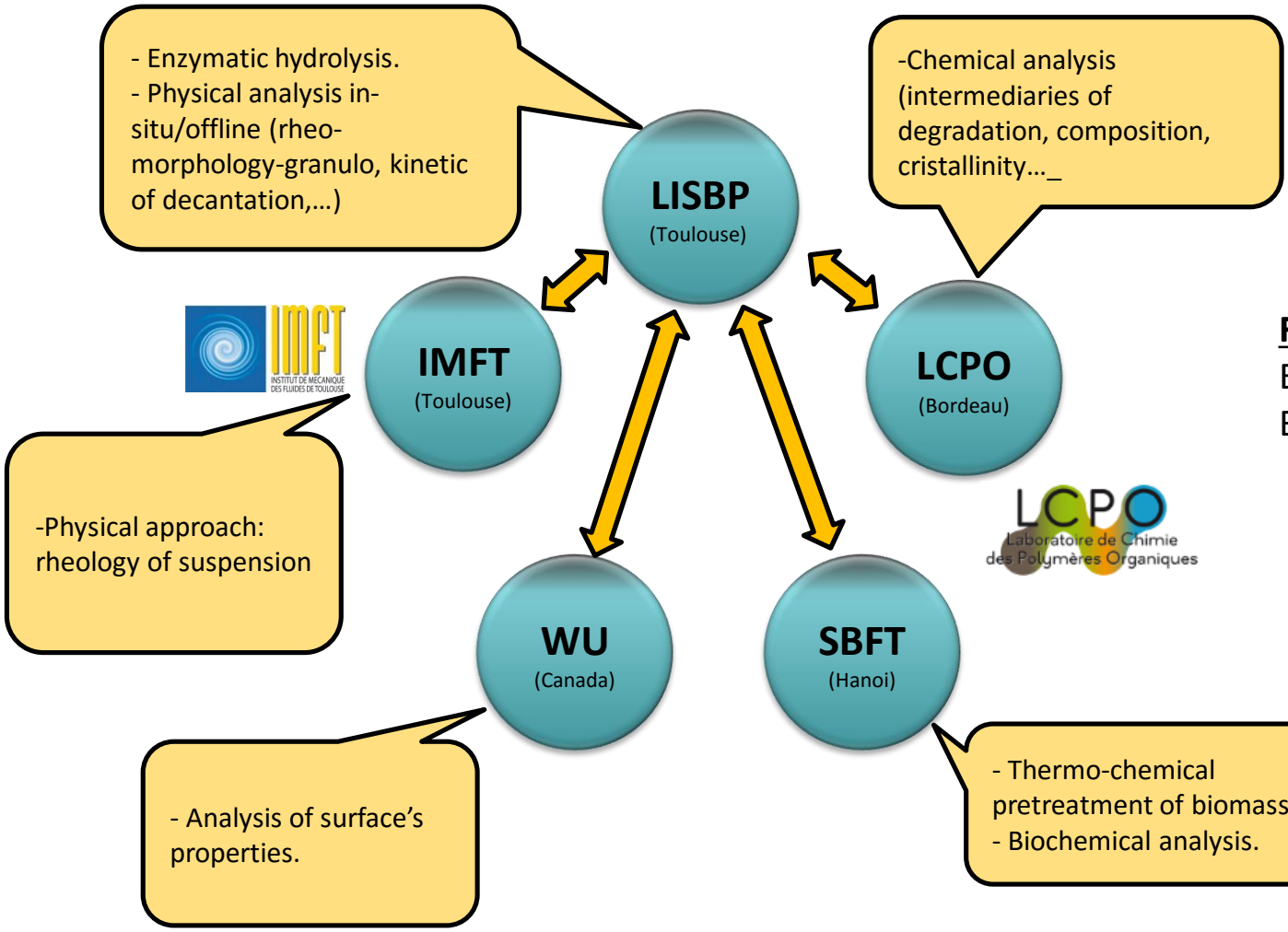
UNIVERSITY OF WATERLOO

IMFT INSTITUT DE MÉCANIQUE DES FLUIDES DE TOULOUSE

UNIVERSITÉ TOULOUSE III PAUL SABATIER Université de Toulouse

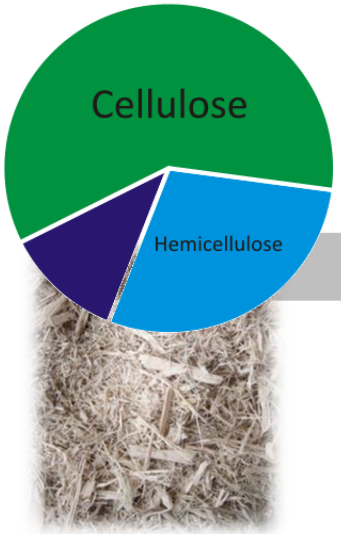
Network & funding



Financial Supports:
Erasmus Mundus - Techno 1
BioAsie – HTMS

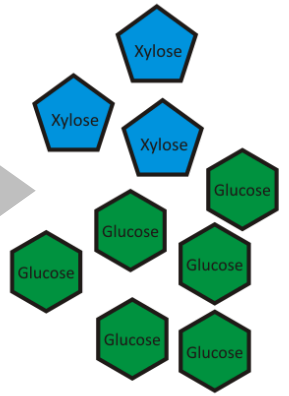
Scientific CONTEXT

Enzymatic hydrolysis of lignocellulosic biomass



Lignocellulolytic enzyme

Cellulase	Hemicellulase
Endo-glucanase (G1)	Endo-xylanase (X1)
Exo-glucanase (G2)	Exo-xylosidase (X2)
β-glucosidase (G3)	Endo-mannanase (M1)
	Exo-mannosidase (M2)



? Properties of suspension

? Destructuration of fibers

Physical limitations

High solid loading
[C6] & [C5] max

Physical approach on enzymatic hydrolysis

Physical approach

- Properties of lignocellulosic fiber suspension (yield stress, rheological behavior, particle size and morphology)

- Evolution of fiber properties during enzymatic hydrolysis

Knowledge

-Lignocellulosic suspension possess **shear thinning properties** (non-Newtonian fluids which have decreased viscosity when subjected to shear strain)

µ, P = f (solid loading) : 4% increase in solid content lead to 5 folds rise in power consumption (Fan et al., 2003; Dunaway et al. 2010; Knutsen et al. 2012)

µ = f (particle size) : at 10% w/w, 50 folds rise in viscosity from particle size 33-75 µm up to 150-180 µm (Dasari and Berson 2007)

Role of **single activities** in the liquefaction : endo-glucanase =dominant (Szijártó et al., 2011)

Physical approach on enzymatic hydrolysis

Physical approach

- Properties of lignocellulosic fiber suspension (yield stress, rheological behavior, particle size and morphology)

Knowledge

-Lignocellulosic suspension possess **shear thinning properties** (non-Newtonian fluids which have decreased viscosity when subjected to shear strain)

μ, P = f (solid loading) : 4% increase in solid content lead to 5 folds rise in power consumption (Fan et al., 2003)

Lack in knowledge

Investigation of pure up to cocktail activities by physical approach: in-situ viscosity, mechanisms of degradation.

➔ My PhD

Materials & Method

Substrate & Experiment parameters

Substrate

Sugarcane bagasse: from sugar factory Nong Cong and Lam Son, VietNam. Organosolv pretreated + extruded.

Filter paper : Whatman n°1, milled

Hard wood paper pulp: from French industrial.

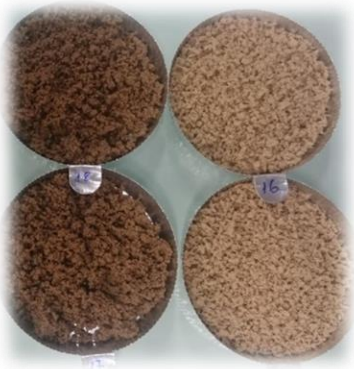
Enzyme

Cellic Ctec2, Novozymes, activity 103FPU/mL at 40°C, pH 4.8

Endo-glucanase: E-CELAN, Megazymes (high purity) – **G1**

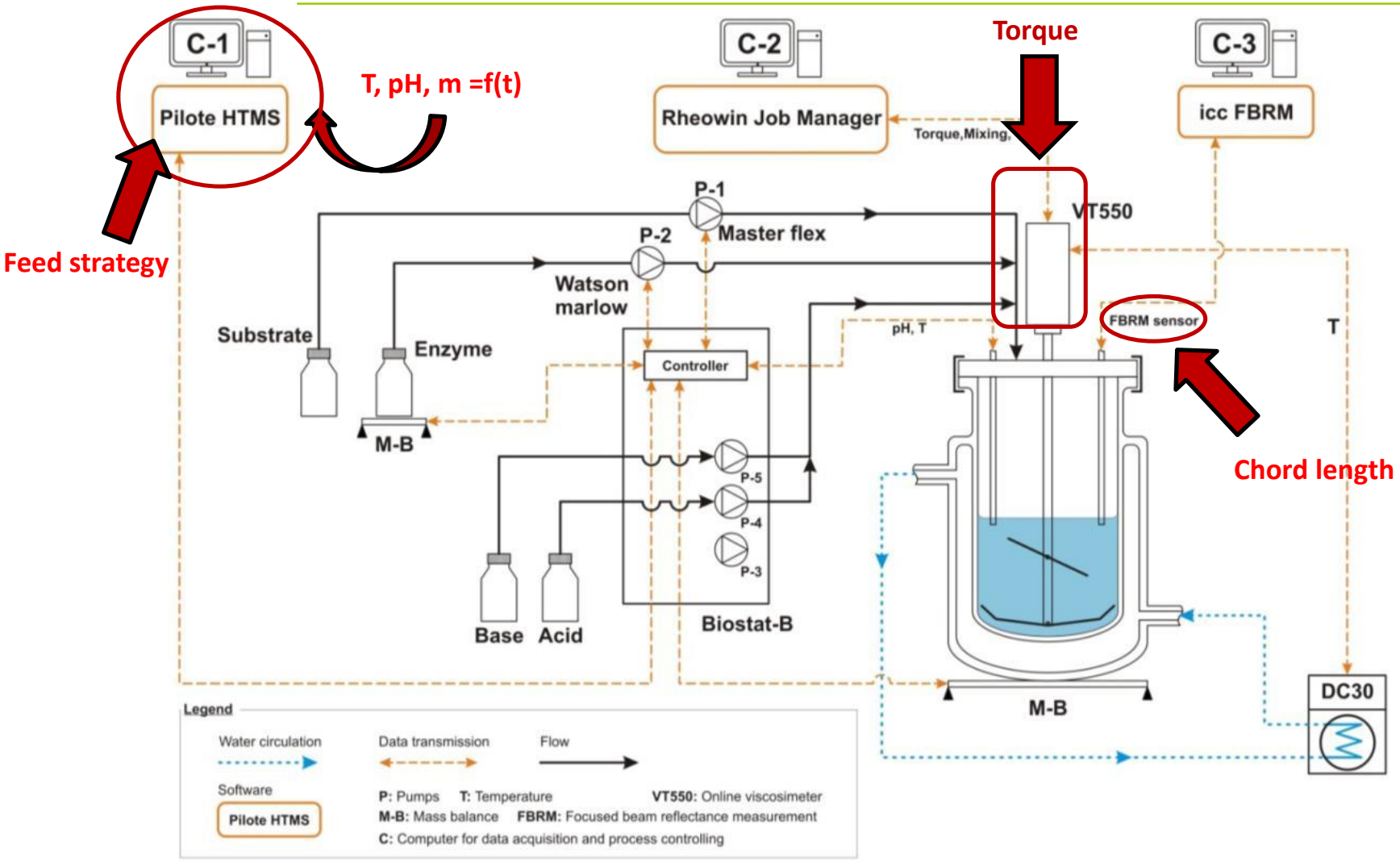
Exo-glucanase: E6412, Sigma – **G2**

β-glucosidase: 49290, Sigma – **G3**



Materials & Method

Experiment setup



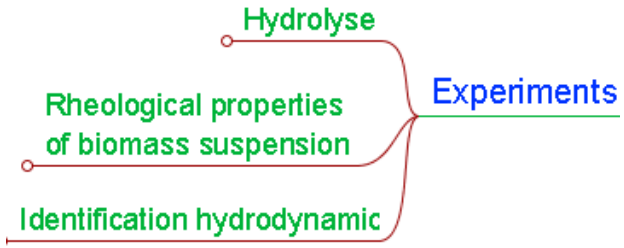
Materials & Method

From raw data to interpreted data

Measurement	Raw data	Interpreted data
In-situ Torque	M=f(t)	Viscosity, rheological behavior
In-situ Chord Length	En(cl), N(cl)=f(t)	Ev, Fv, En, Fn, dSE
Ex-situ rheology	G', G''	Viscosity, yield stress
DLS	Ev(dSE), d[4,3]	Ev(dSE), Fv(dSE), d[4,3]
Morphologi	Particle sharpe & size	Ev(dCE), Particle sharpe & size
Decantation kinetic	Settling velocity	
HPLC	Mono to di-saccharides	Hydrolysis yield

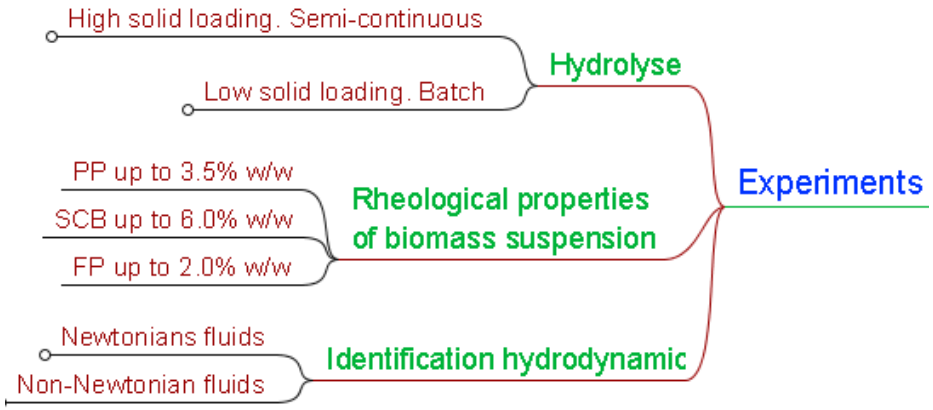
Research STRATEGY

Working diagram



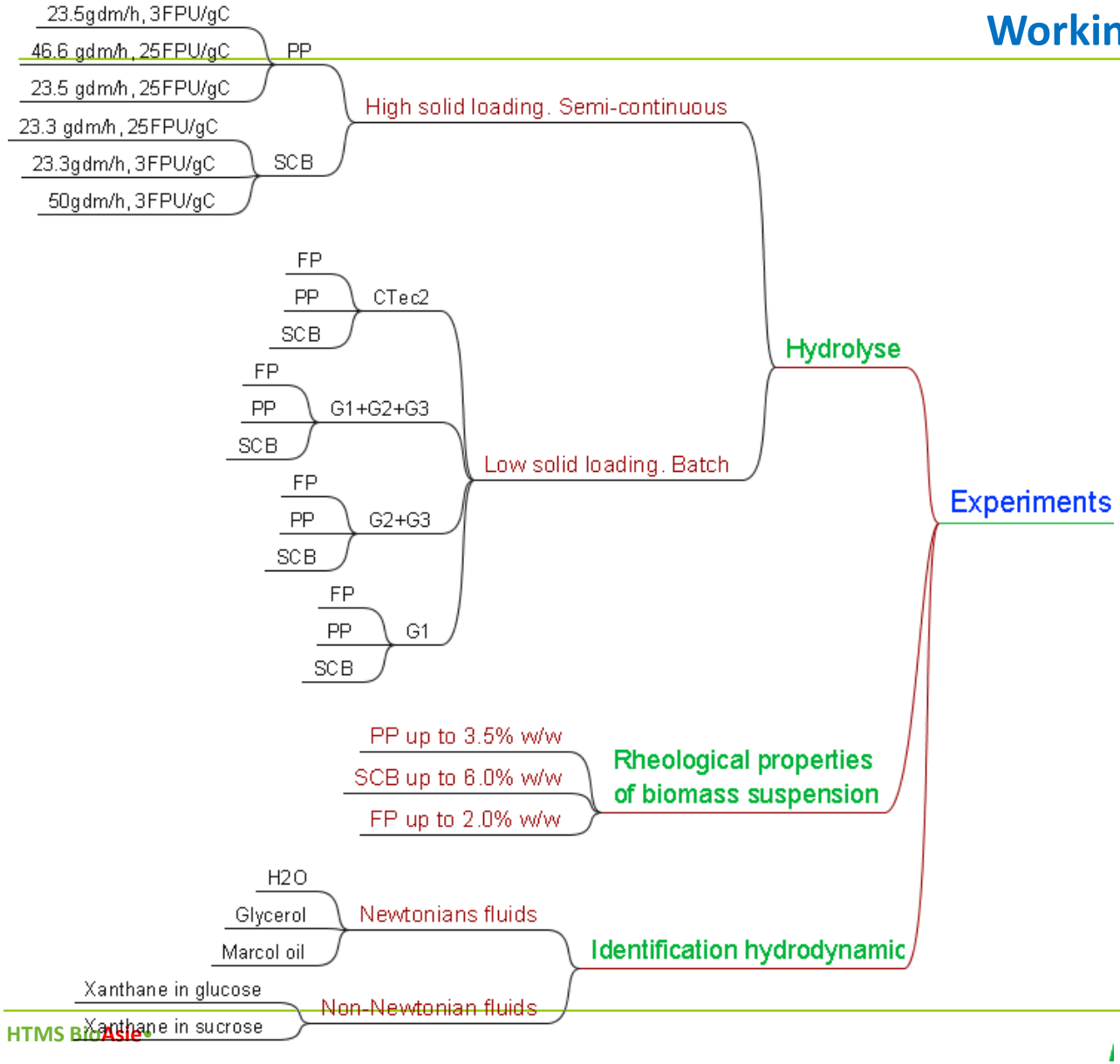
Research STRATEGY

Working diagram



Research STRATEGY

Working diagram



Research STRATEGY

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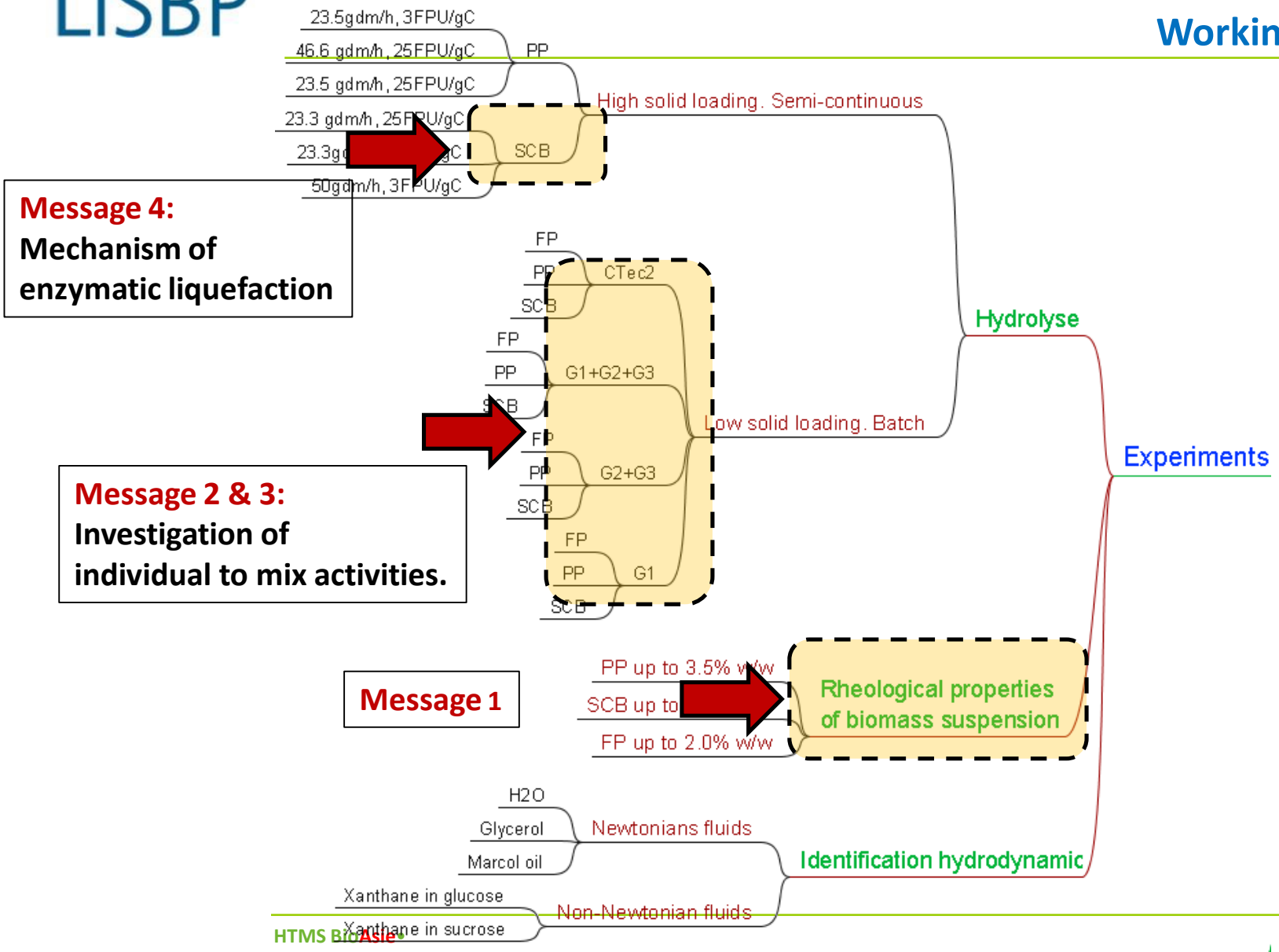
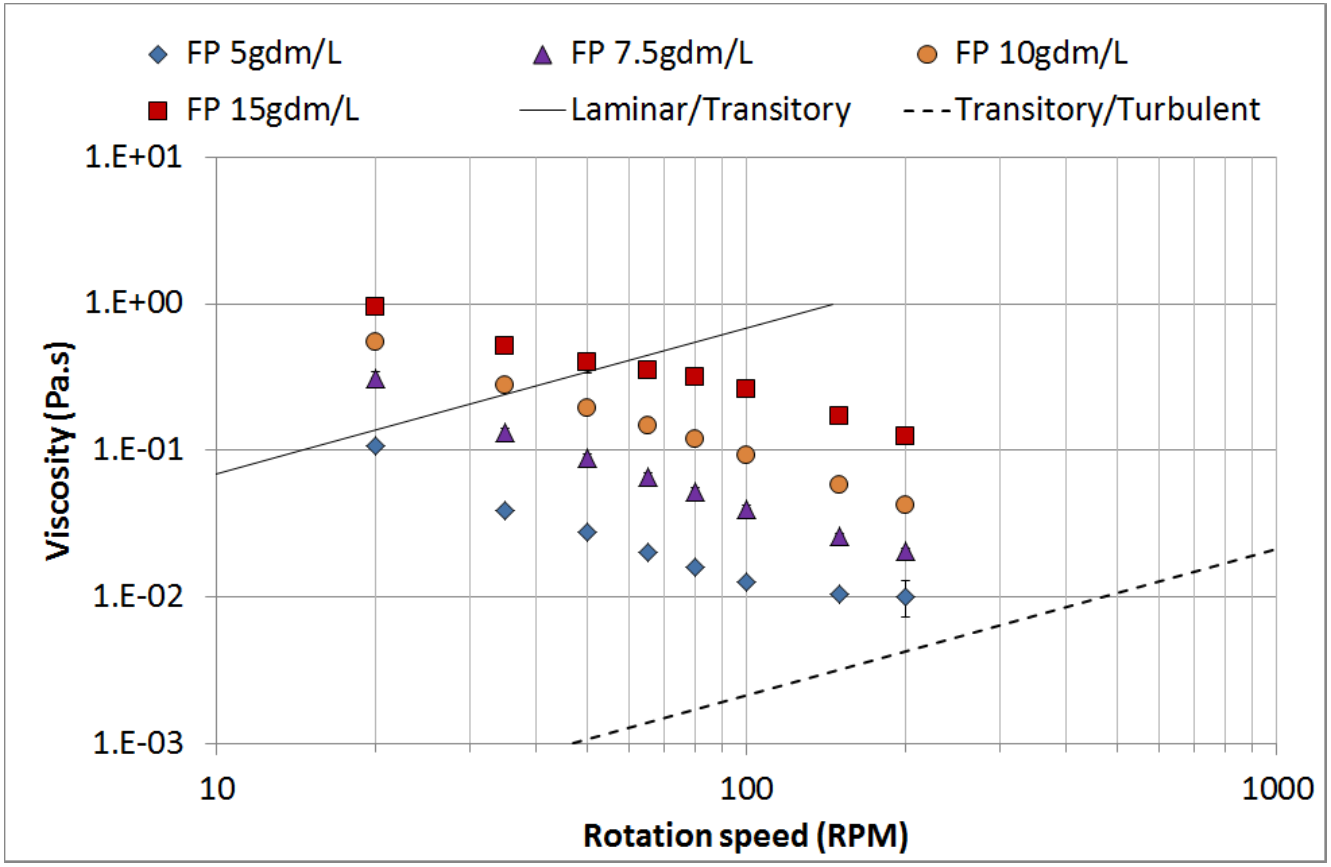
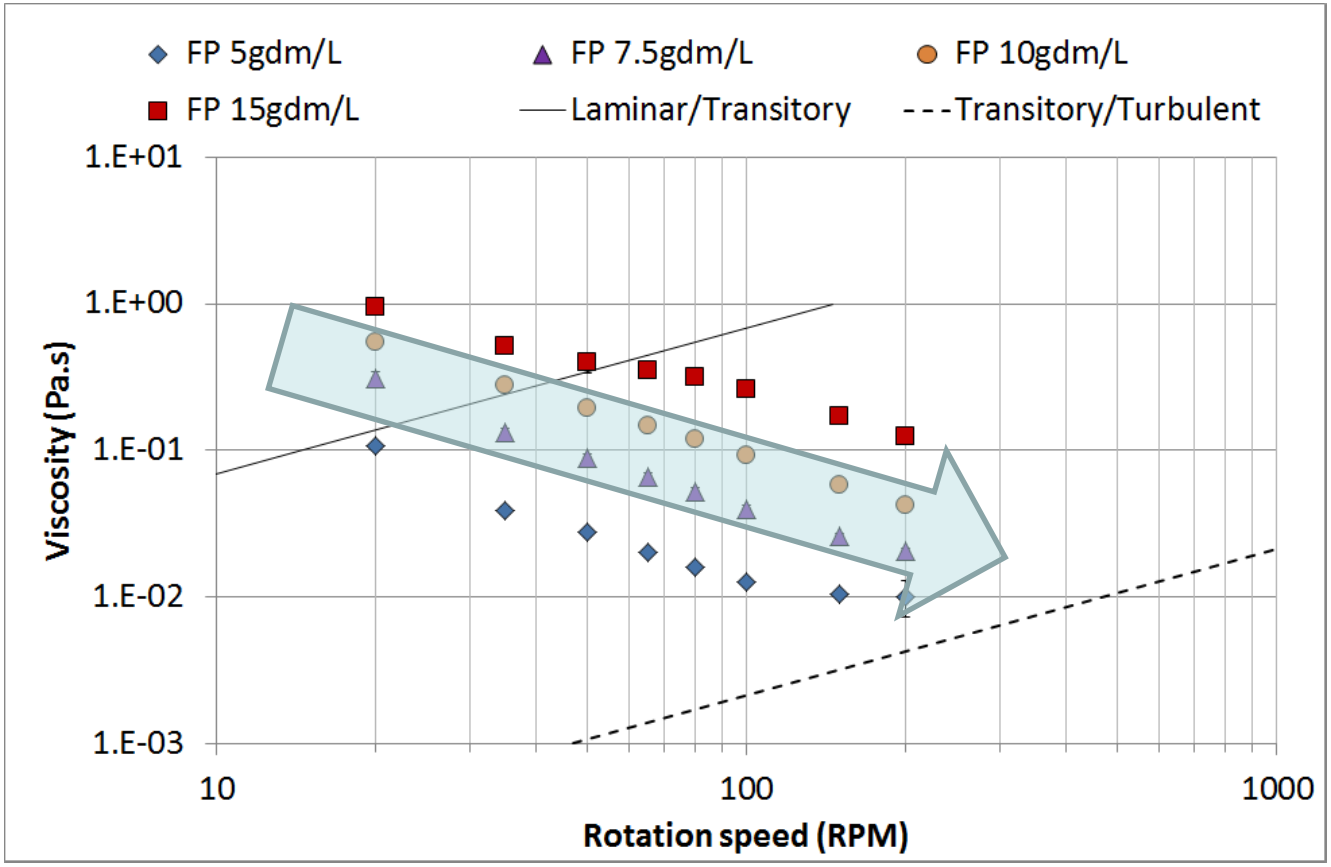


Substrate properties - FP



Suspension viscosity at different mixing rate
Substrate: **filter paper**

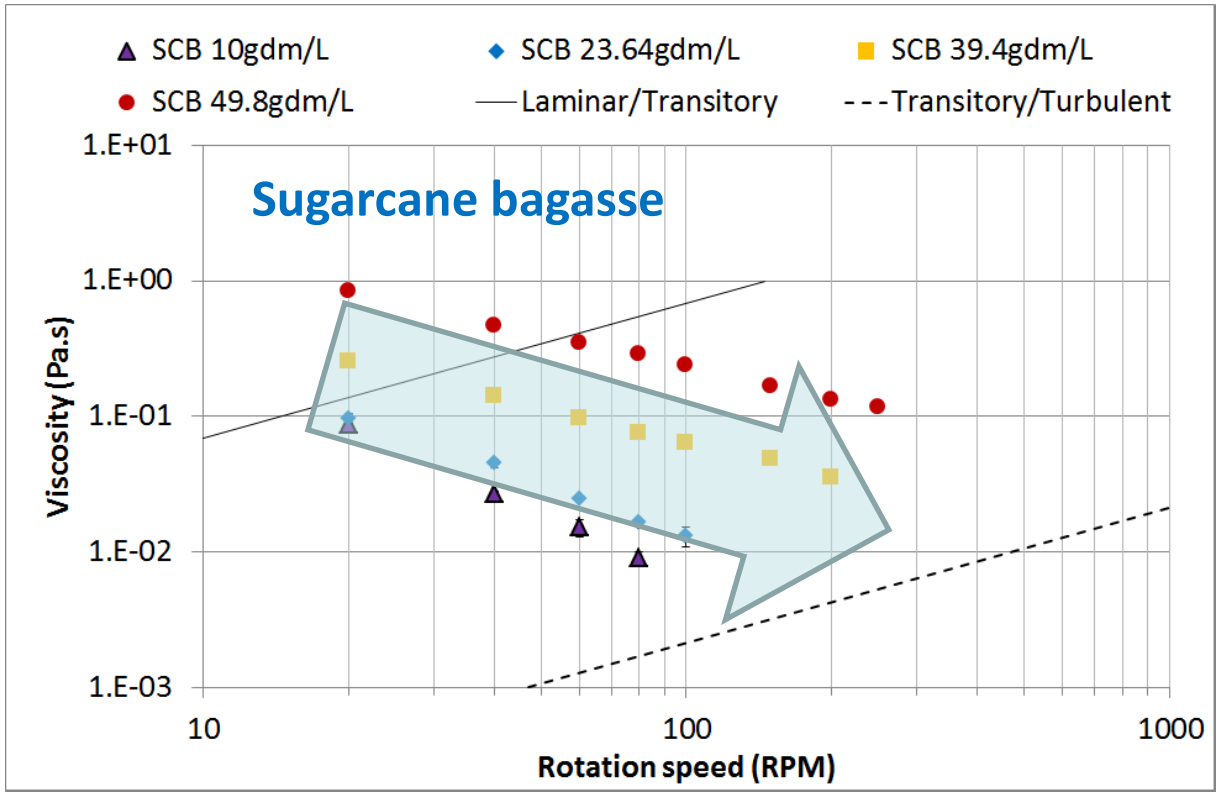
Substrate properties - FP



Suspension viscosity at different mixing rate
Substrate: **filter paper**

Suspension viscosity decreased as the mixing rate increased
-> shear-thinning properties

Substrate properties – SCB and PP



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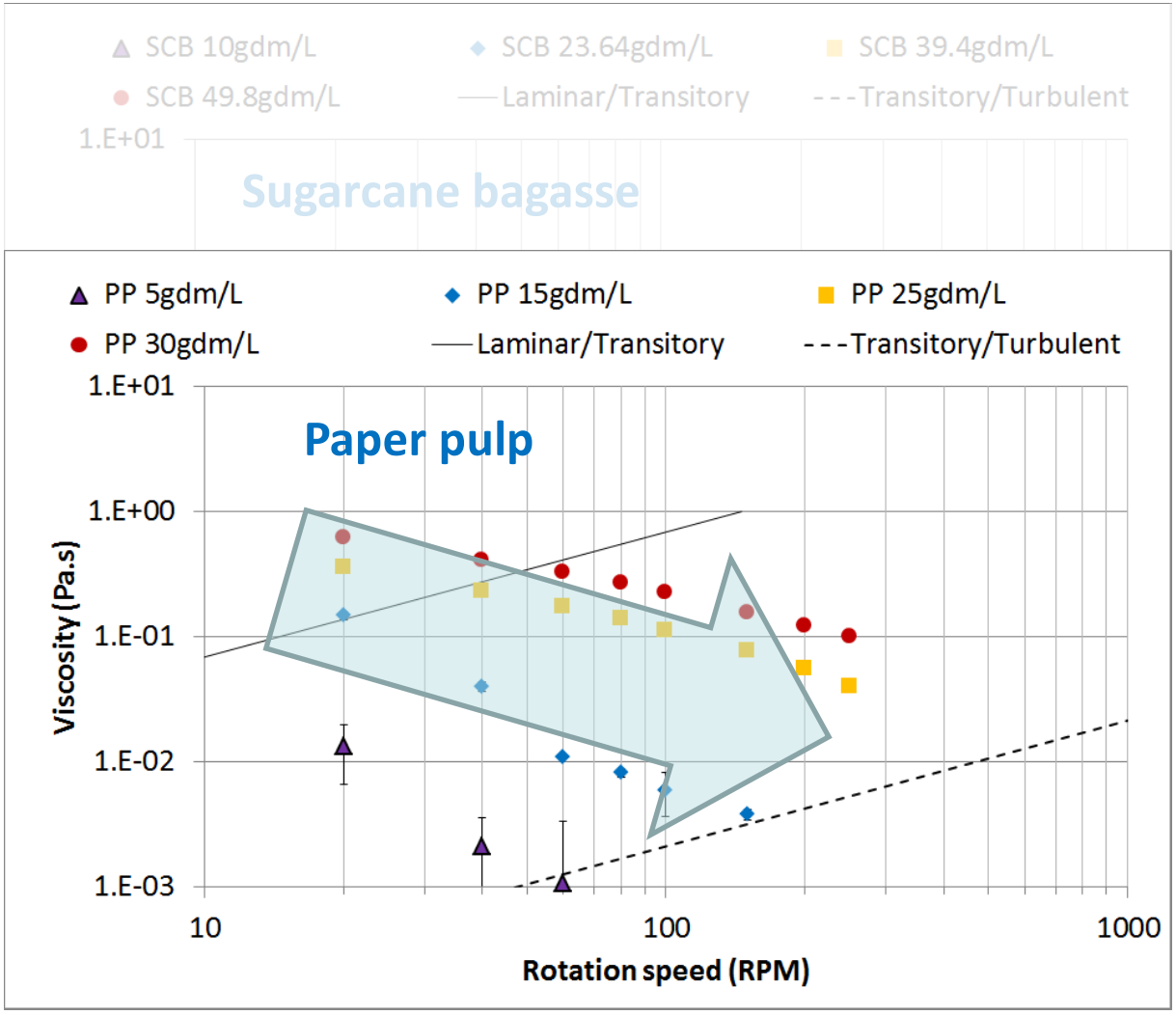
Similar behavior on **sugarcane bagasse** …

Substrate properties – SCB and PP

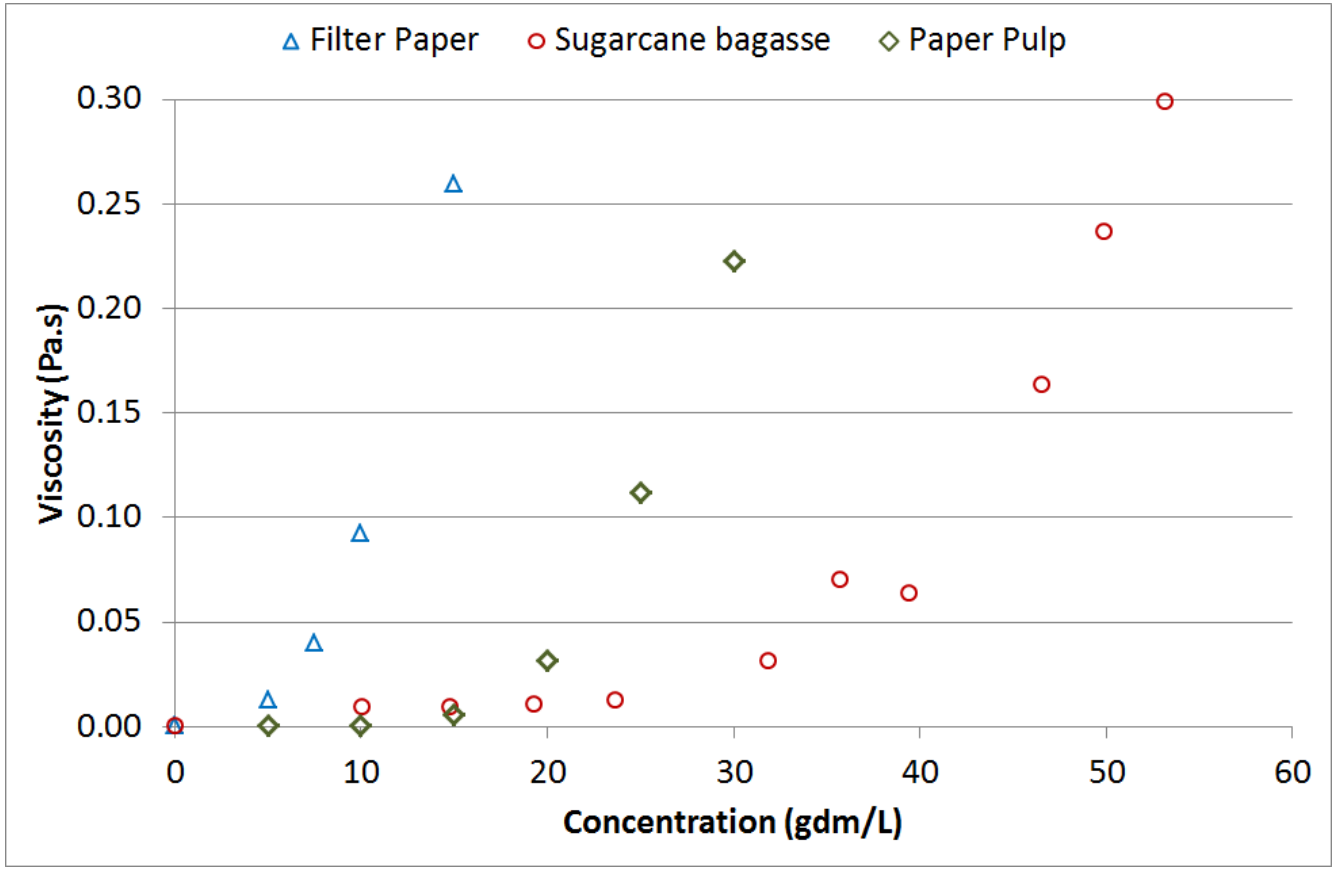


Suspension viscosity at different mixing rate
Substrate: **filter paper**

Suspension viscosity decreased as the mixing rate increased
-> shear-thinning properties

Similar behavior on **sugarcane bagasse** …
and on **paper pulp**

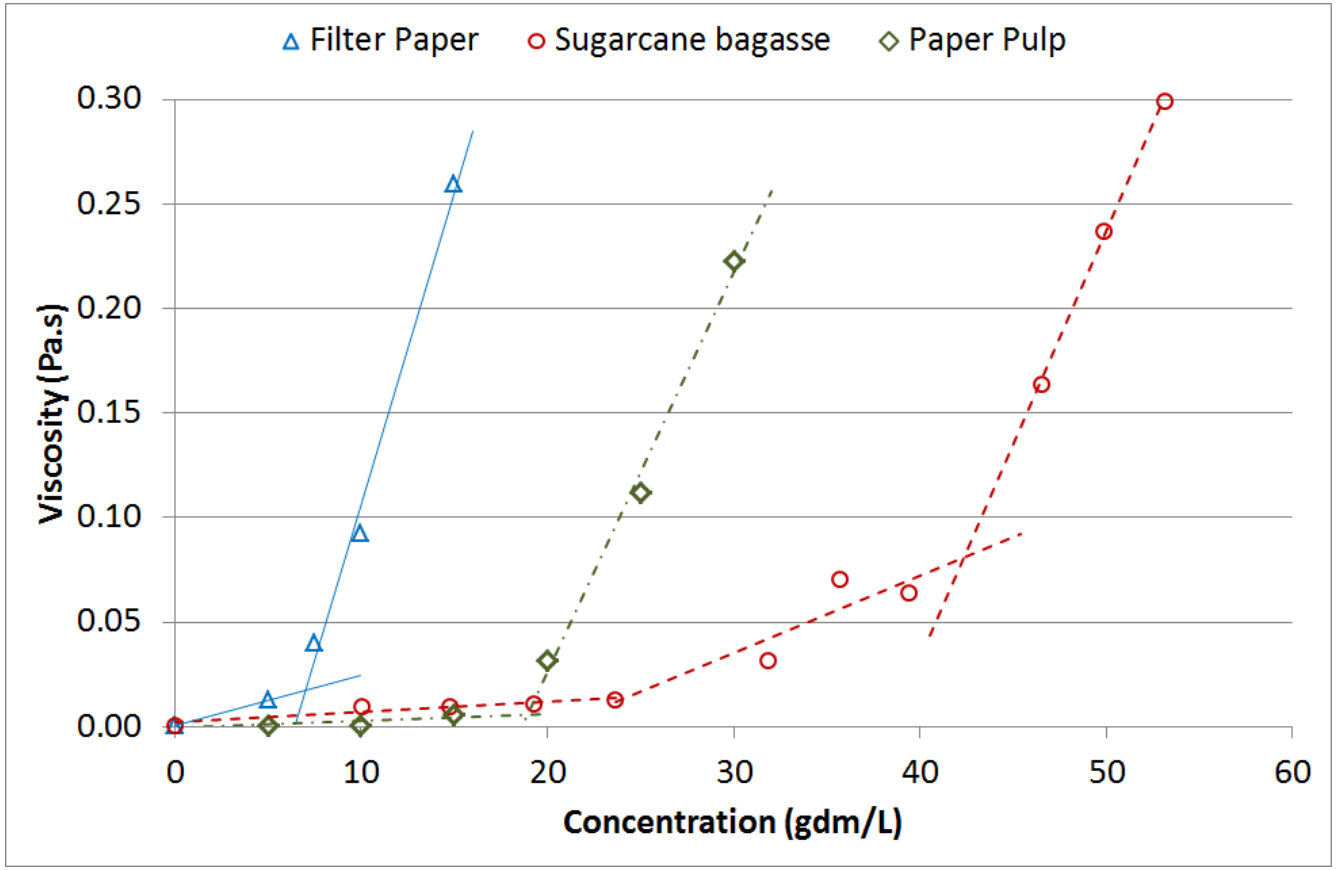
Substrate properties



Viscosity at 100 rpm of SCB, FP and PP suspensions in relation with **biomass concentration**

RESULTS & DISCUSSION

Substrate properties

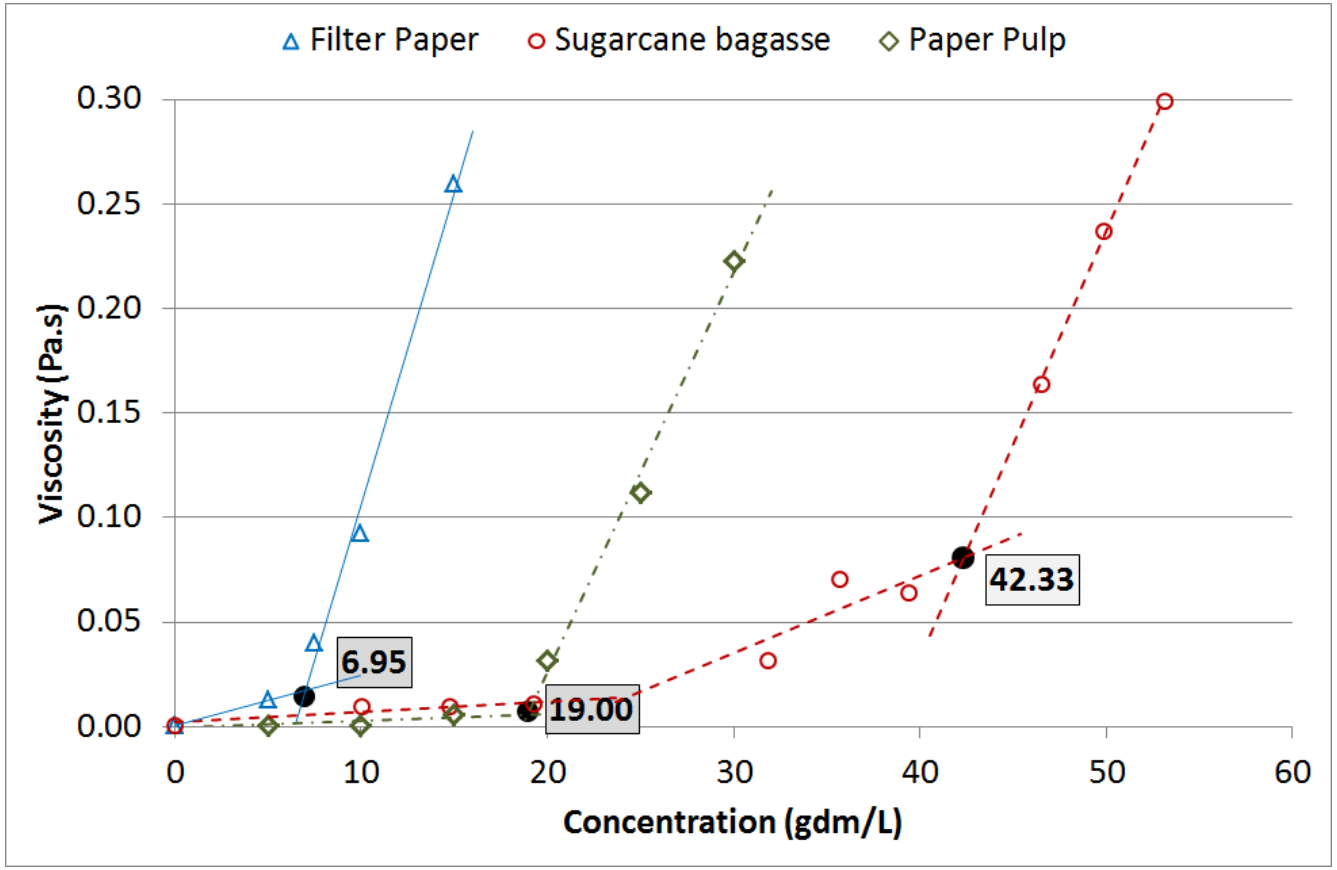


Viscosity at 100 rpm of SCB, FP and PP suspensions in relation with **biomass concentration**

Viscosity rise as **biomass concentration** increased

RESULTS & DISCUSSION

Substrate properties

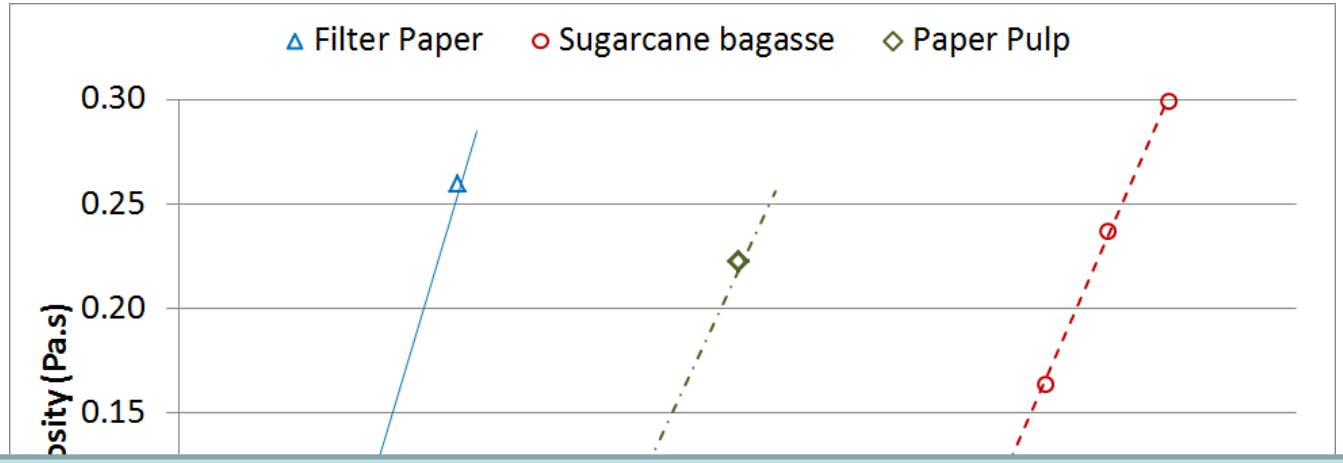


Viscosity at 100 rpm of SCB, FP and PP suspensions in relation with **biomass concentration**

Viscosity rise as **biomass concentration** increased

Identification of substrate's **critical concentration**

Substrate properties



Viscosity at 100 rpm of SCB, FP and PP suspensions in relation with **biomass concentration**

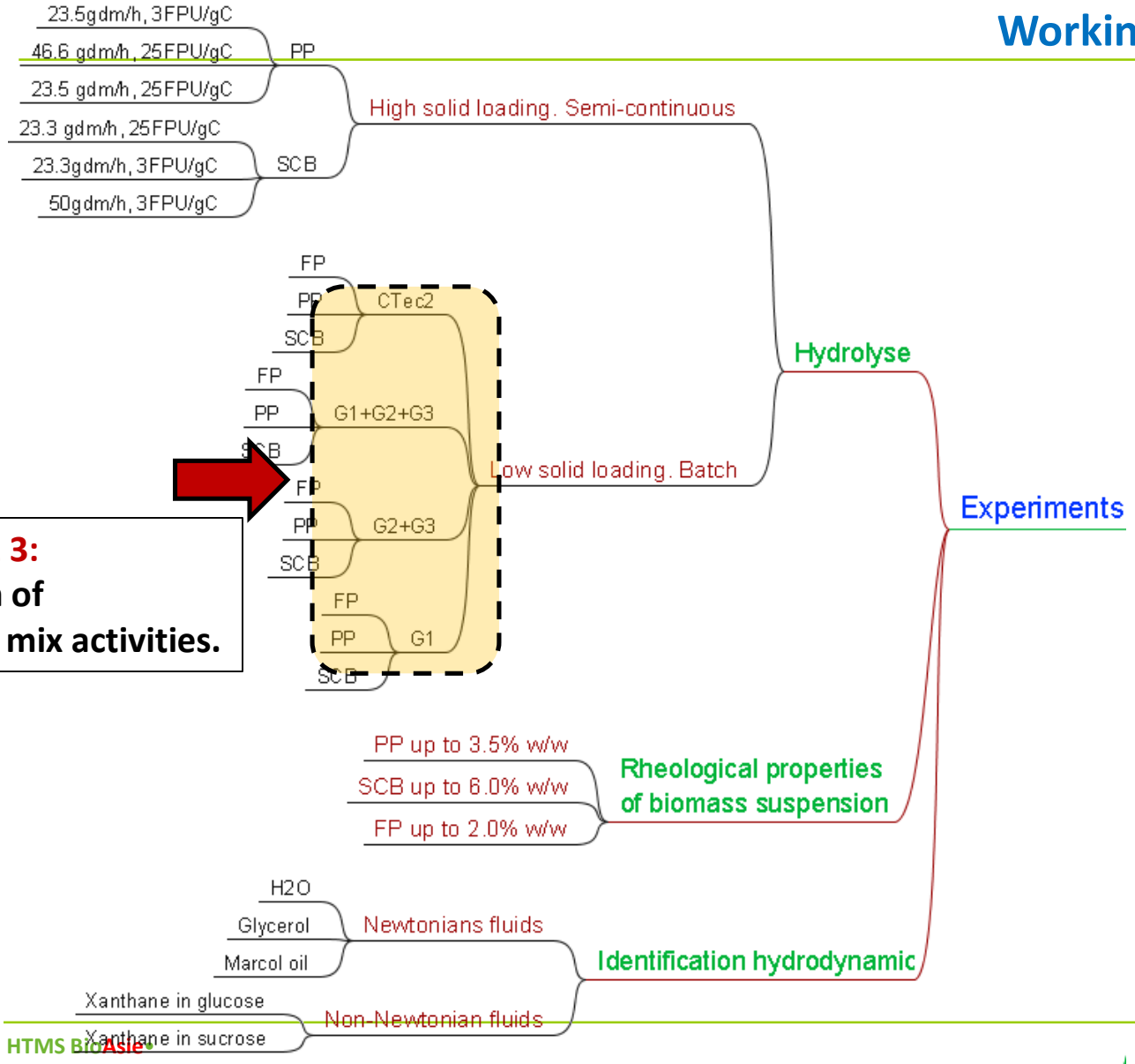
Viscosity rise as **biomass concentration** increased

Message 1

- All studied suspension behaved as shear-thinning fluid.
- Suspension viscosity = f(solid loading).
- Identification of critical concentration points.

Research STRATEGY

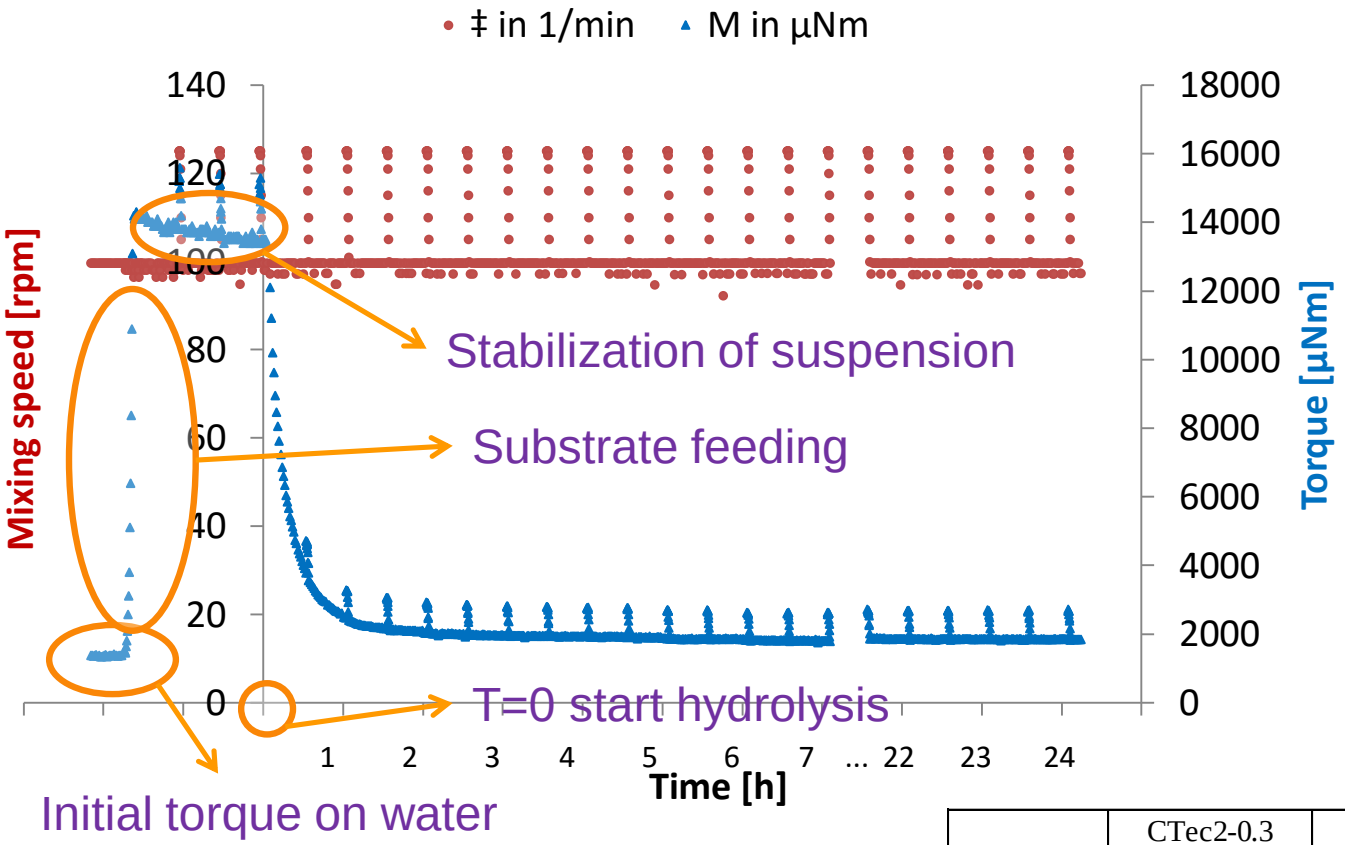
Working diagram



Message 2 & 3:
Investigation of individual to mix activities.

Materials & Method

Experiment setup – batch process



Hydrolysis parameters

-Mixing speed:
- 100 rpm for 28 min
- 125 rpm for 1 min
- speed-down to 100 rpm
- repeat until the end

-pH: 4,8

-Temperature: 40°C

-Substrate: 1,5-3% w/v

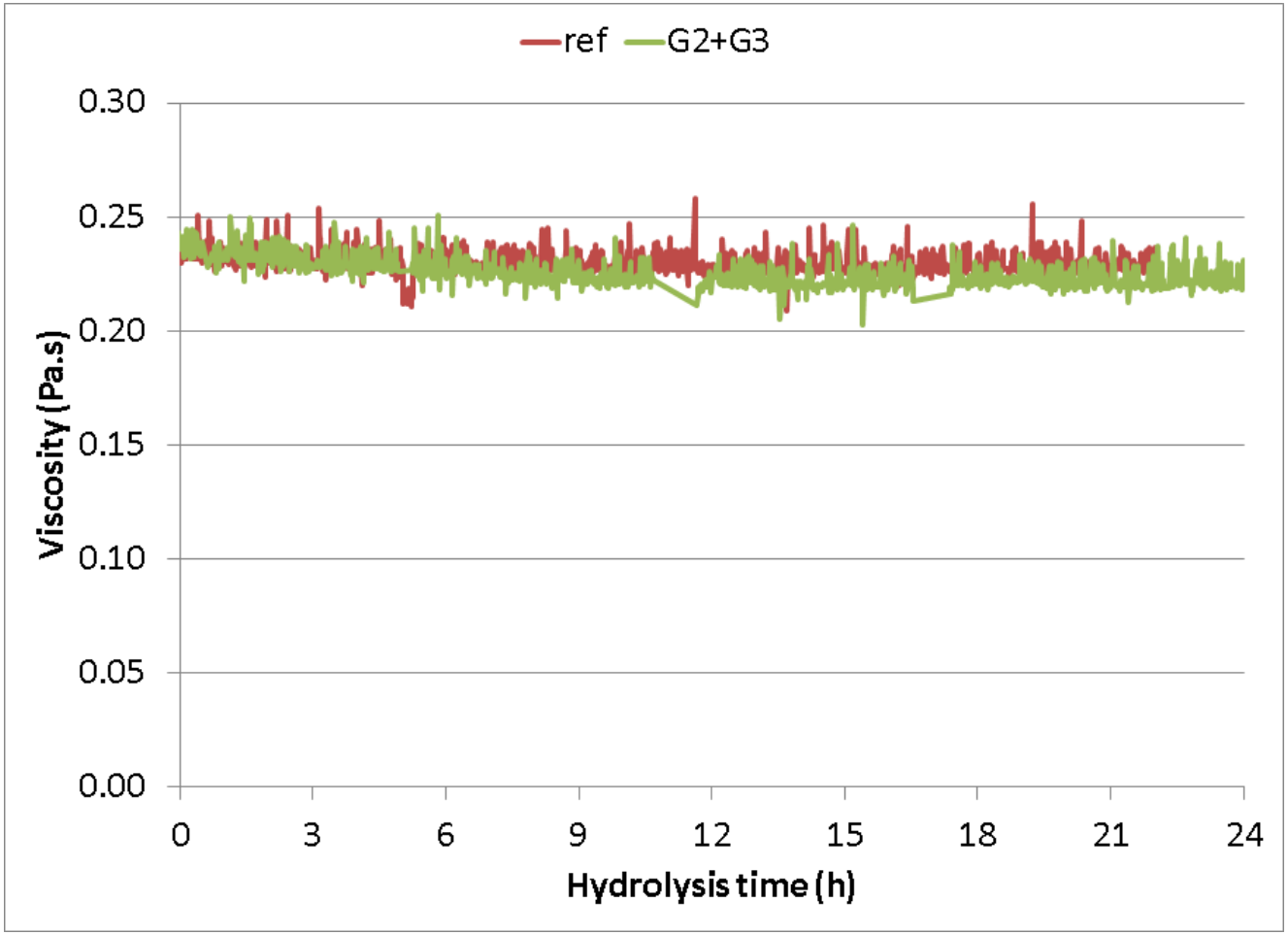
Sampling at 0-1-2-3-6-12-18-24h

Enzyme dosage

	CTec2-0.3	G1	G2+G3	G1+G2+G3
FPU	0.30	/	/	
CMCU	2.42	2.42	/	2.42
AVCU	0.30	/	3.00	3.00
CBU	10.95	/	0.50	0.50

Pure activities contribution for liquefaction

Filter paper 15gdm/L



Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **no contribution**

Pure activities contribution for liquefaction

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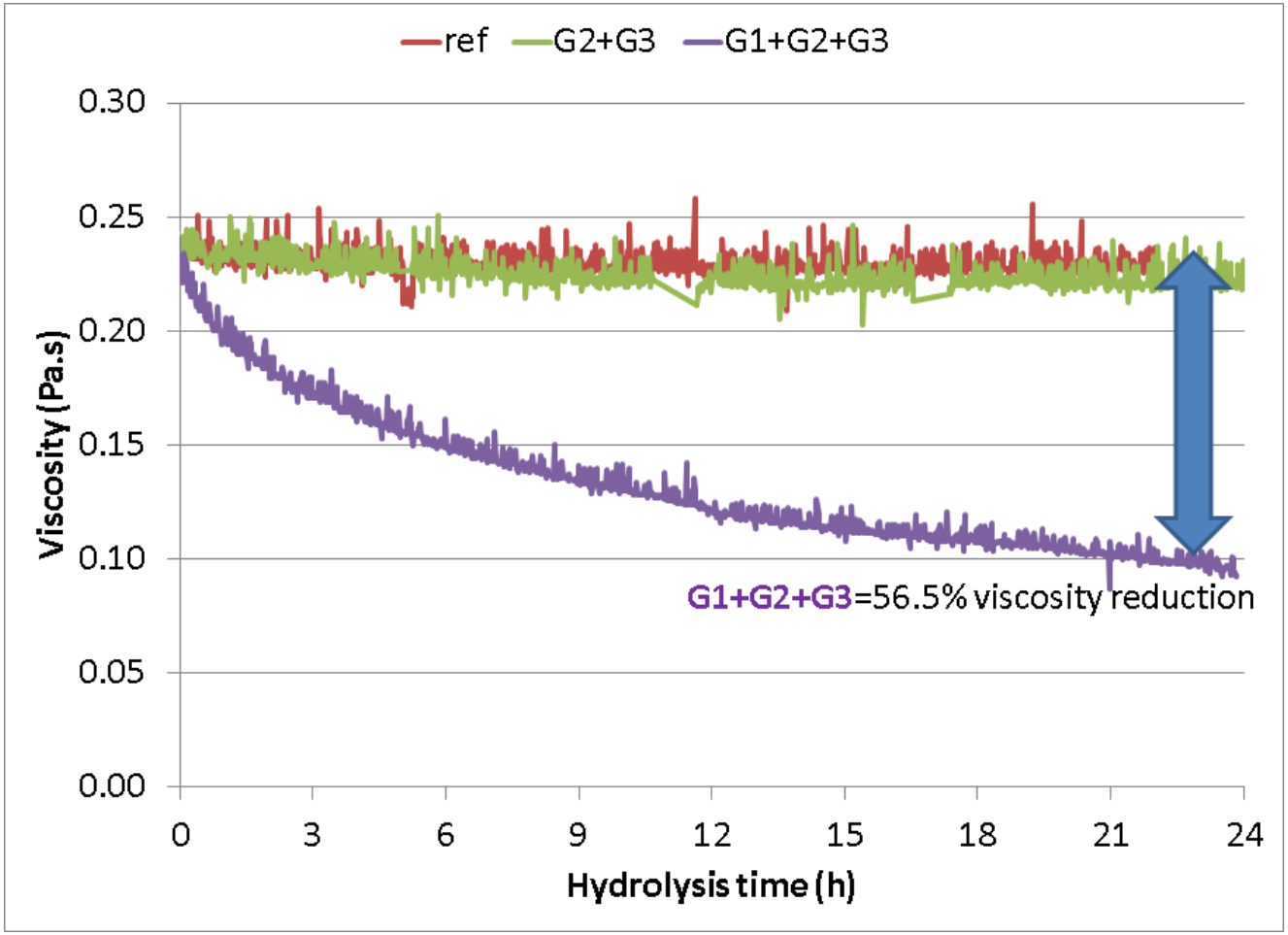

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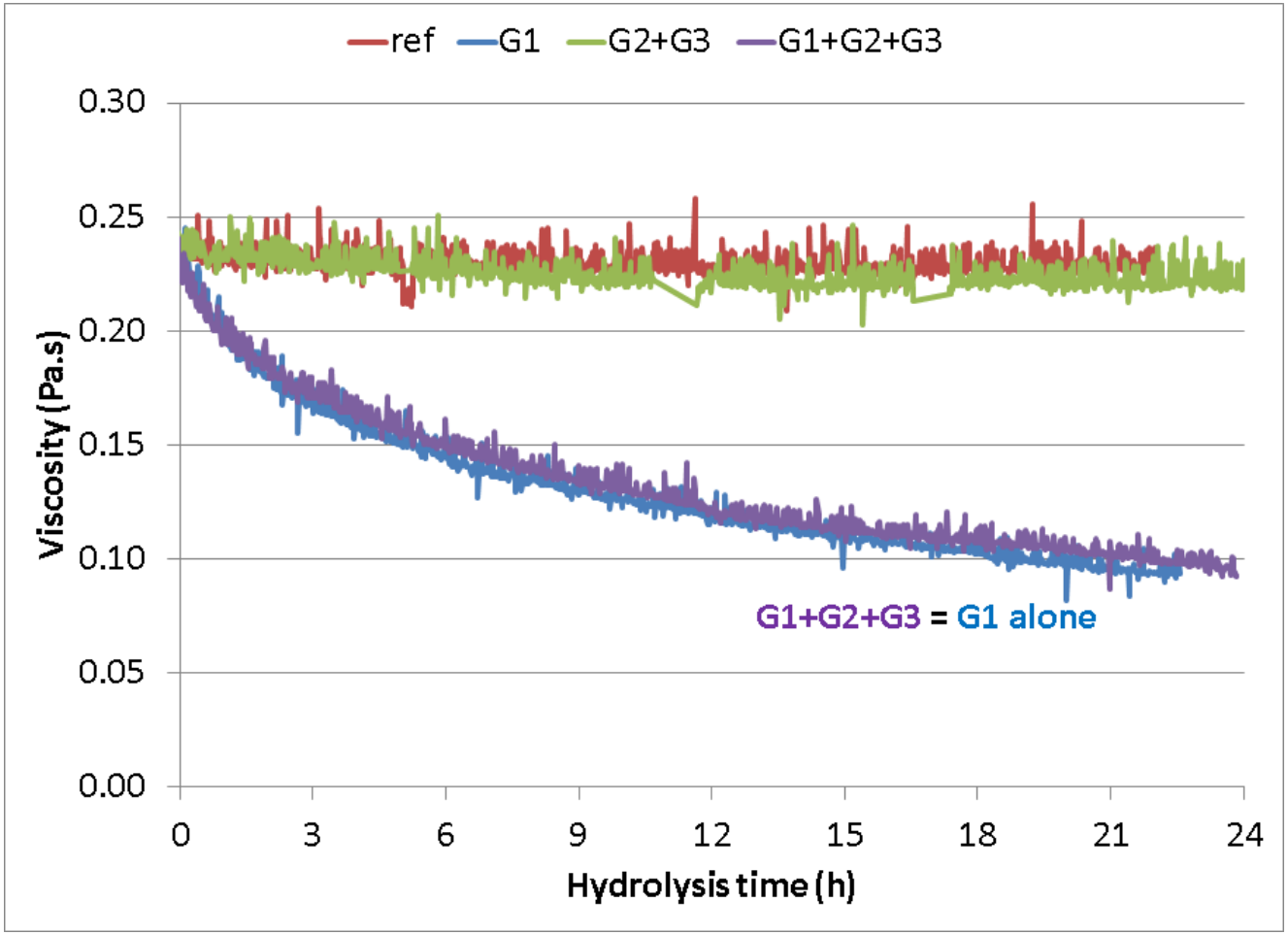


Mixing rate 100rpm

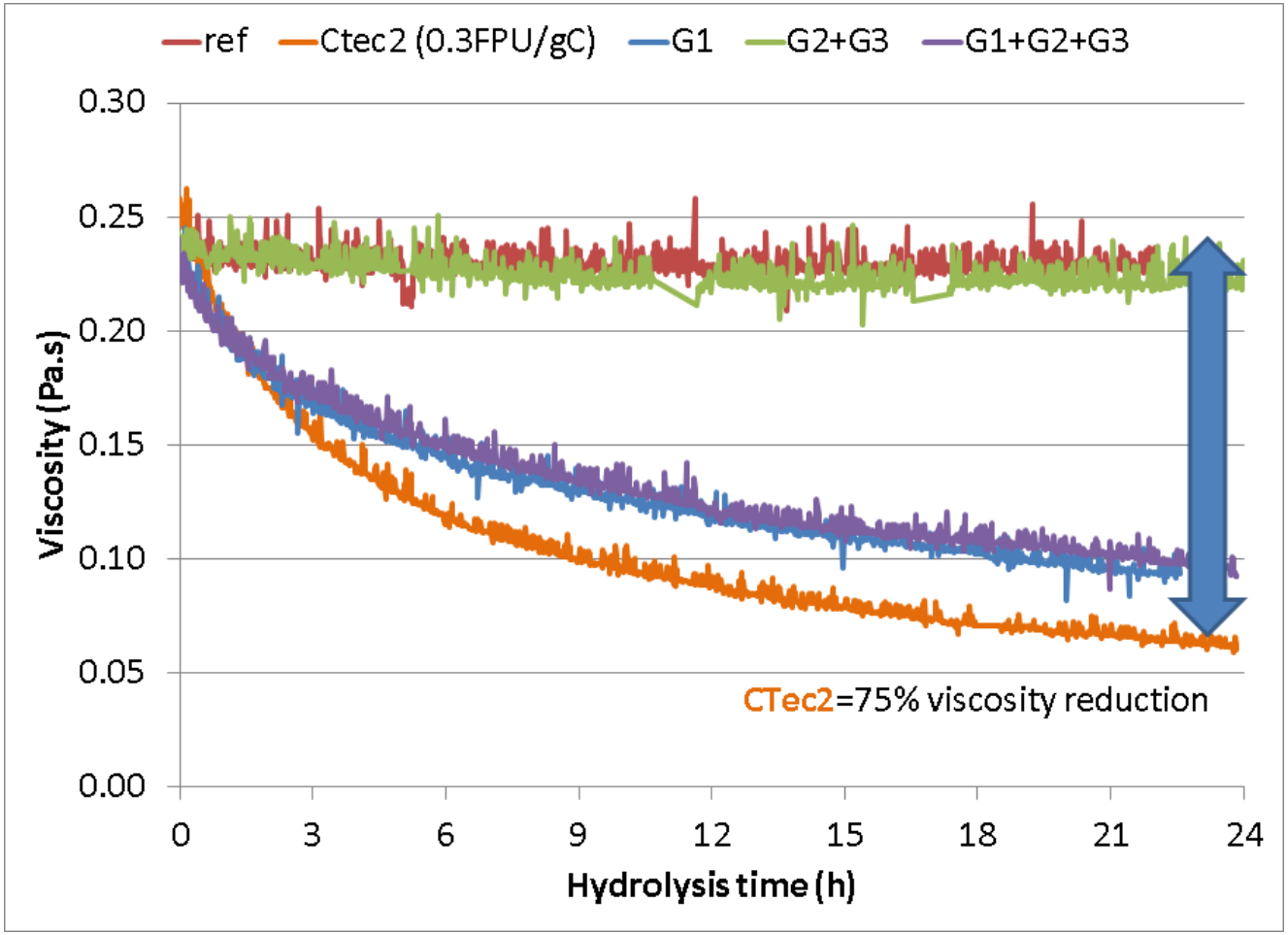
Ref: no enzyme

G2+G3 (exo-glucanase & β-glucosidase) = **no contribution.**

G1 (endo-glucanase) = predominant role.

Pure activities contribution for liquefaction

Filter paper 15gdm/L



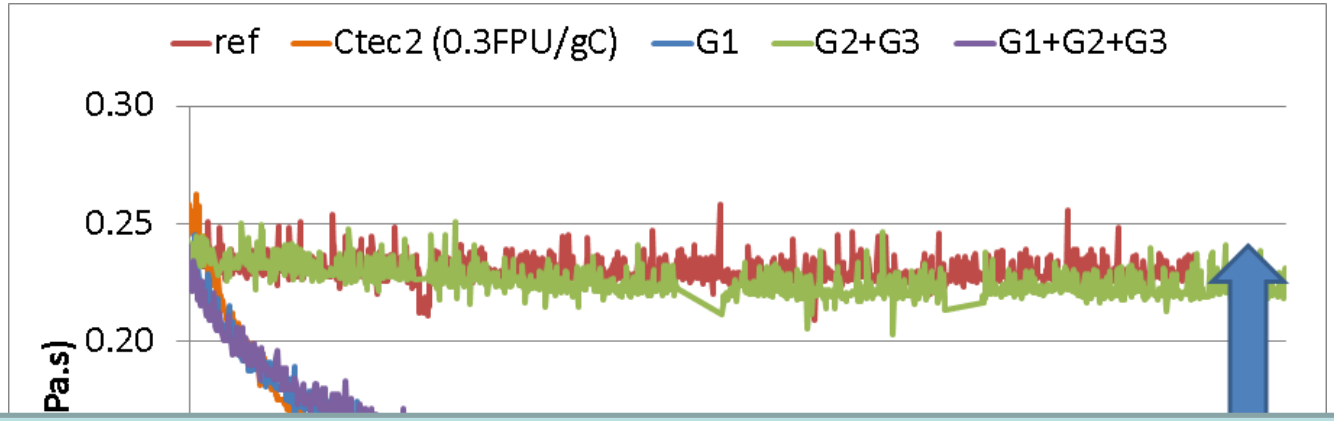
Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **no contribution.**

G1 (endo-glucanase) = predominant role.

CTec2 = better performance !
Enzyme synergist ?.

Pure activities contribution for liquefaction

Filter paper 15gdm/L



Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **no contribution.**

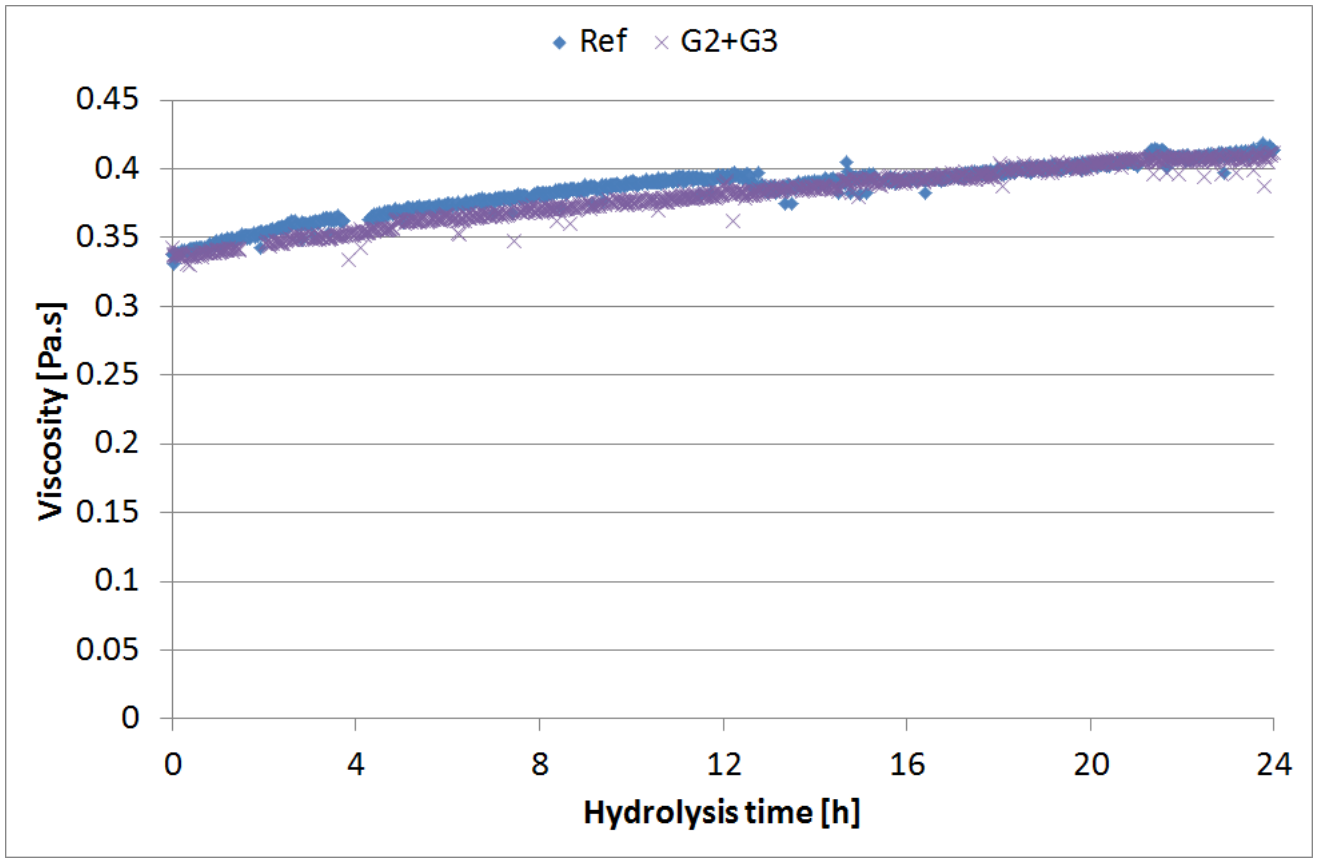
G1 (endo-glucanase) =

Conclusions on filter paper:

- Liquefaction: G2+G3=almost no effect, G1=predominant role.
- G1+G2+G3 no synergy observed
- Ctec2 showed better performance. Hypothesis on enzyme synergist ???

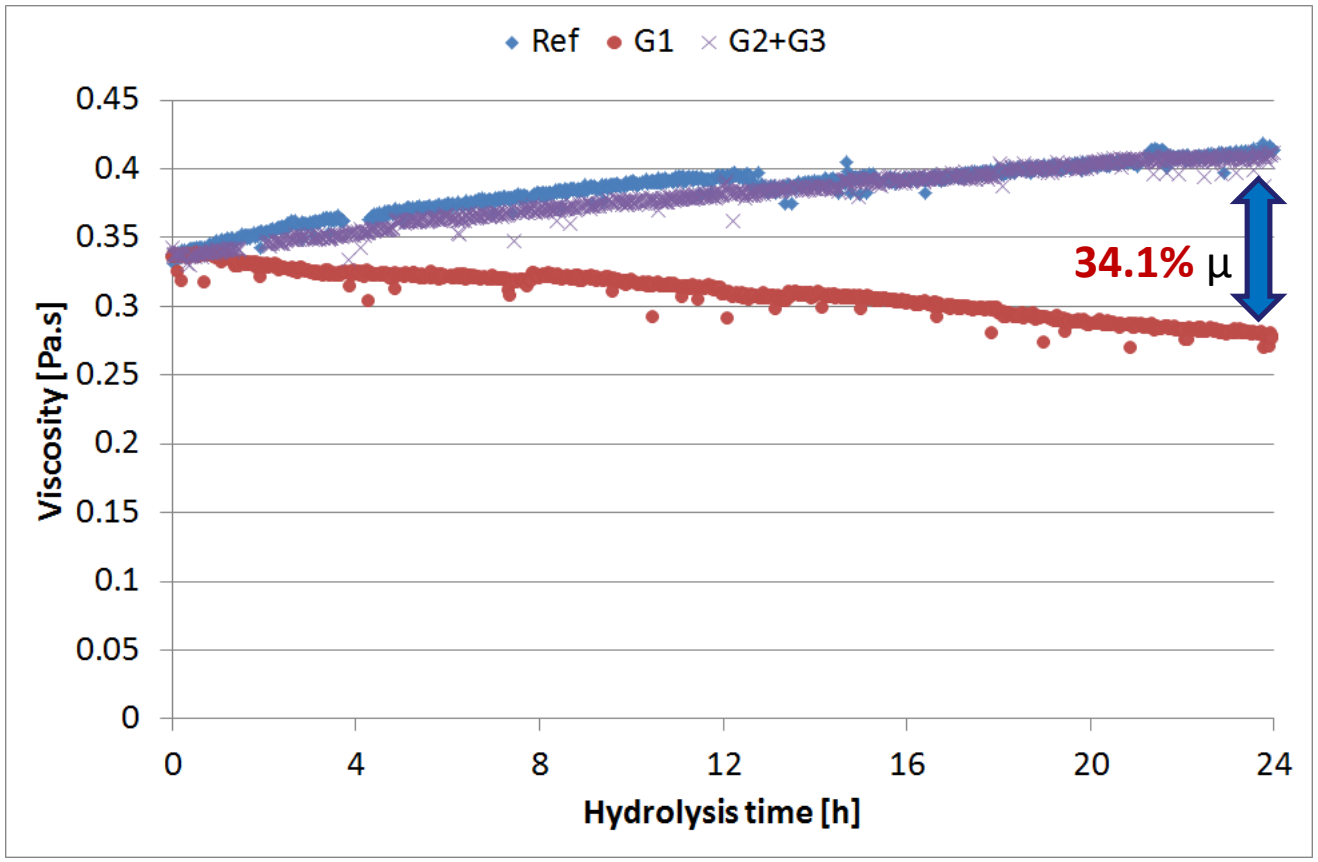
Question: how enzymes act on complex lignocellulosic suspension ?

Results on hard wood paper pulp



Paper pulp 30gdm/L
Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **no contribution.**

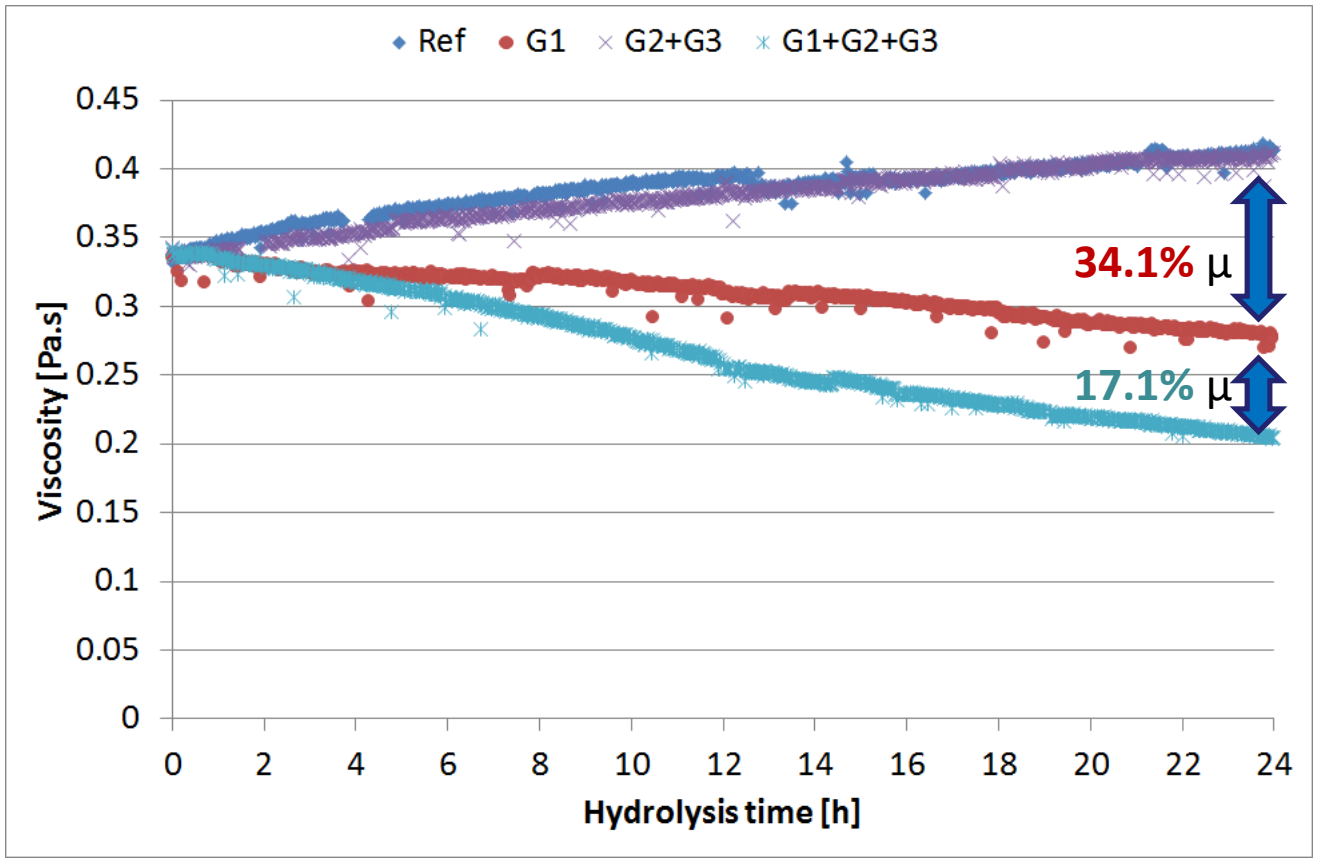
Results on hard wood paper pulp



Paper pulp 30gdm/L
Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **no contribution.**

G1 (endo-glucanase) = **34.1%** reduction in µ.

Results on hard wood paper pulp



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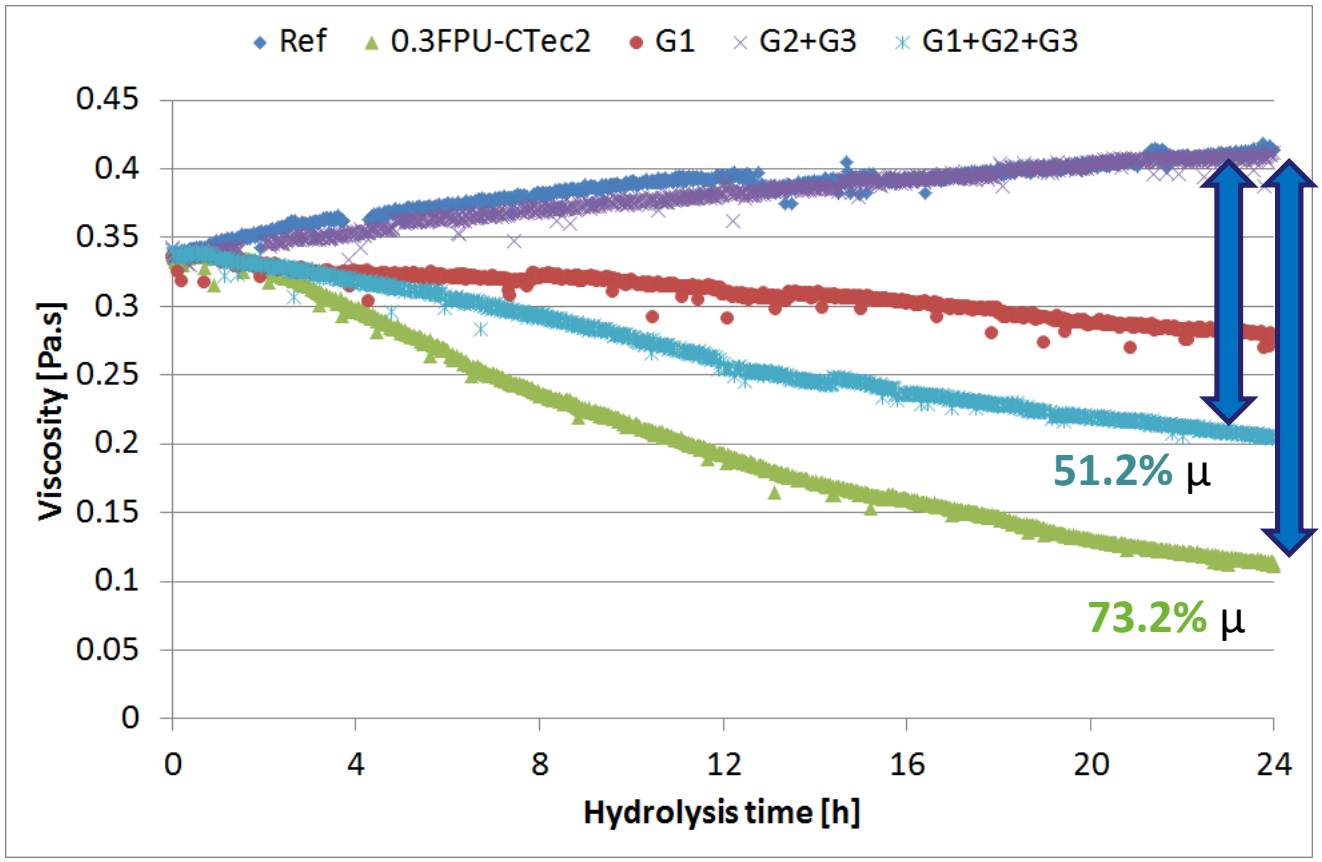
G1 (endo-glucanase) = **34.1%** reduction in µ.

Enzyme synergyof **G1+G2+G3** **51.2%** reduction in µ = 1.5fold increase vs **G1** alone.

Results on hard wood paper pulp



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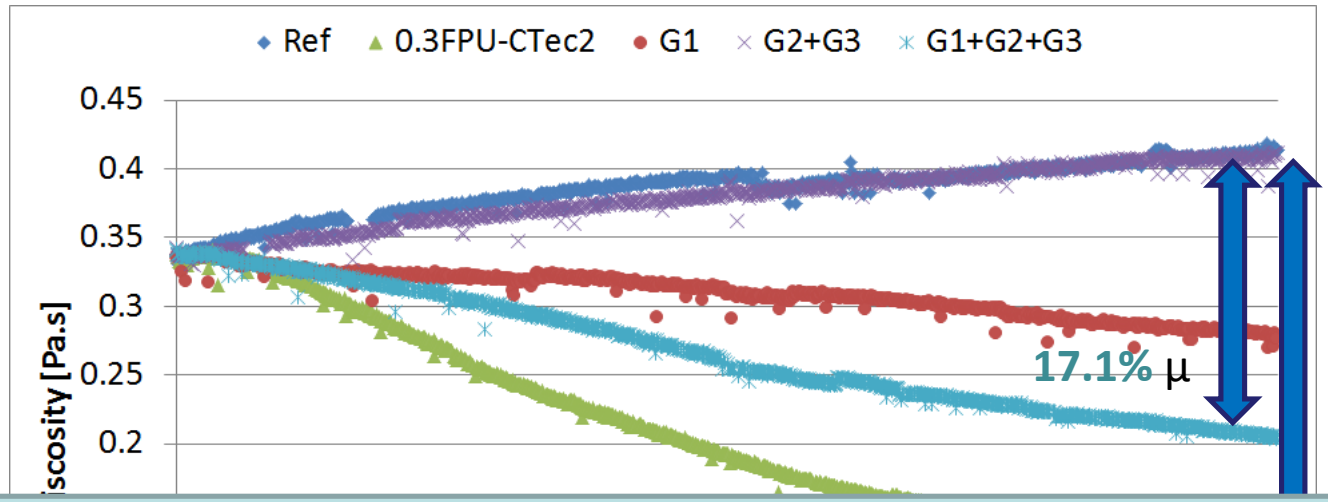
G1 (endo-glucanase) = **34.1%** reduction in µ.

Enzyme synergy of **G1+G2+G3** **51.2%** reduction in µ = 1.5fold increase vs **G1** alone.

Ctec2 >> **G1+G2+G3** **73.2%** reduction in µ.

Results on hard wood paper pulp



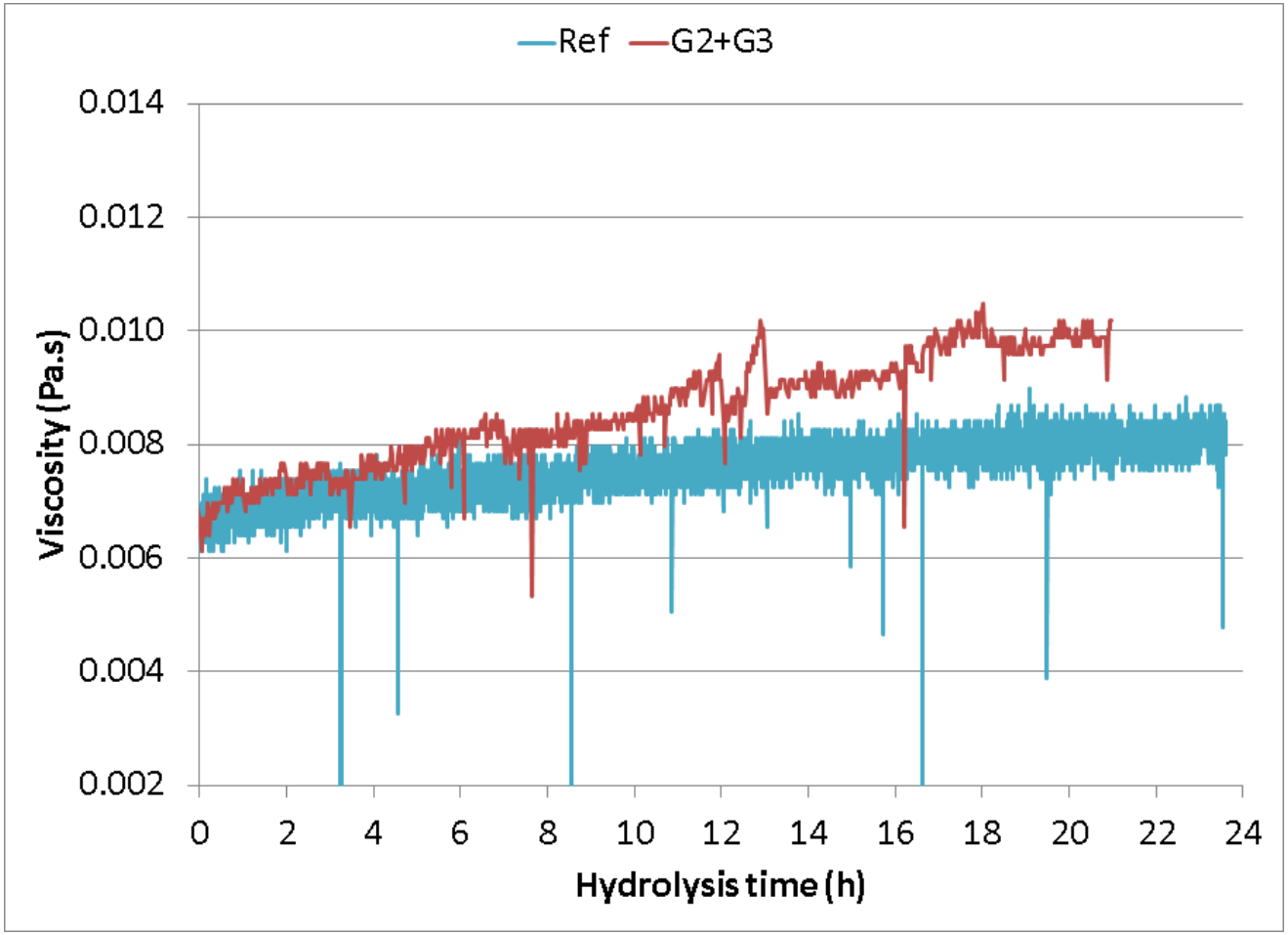
Paper pulp 30gdm/L
Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **no contribution.**

G1 (endo-glucanase) = **20.6%** reduction in µ.

Conclusions paper pulp

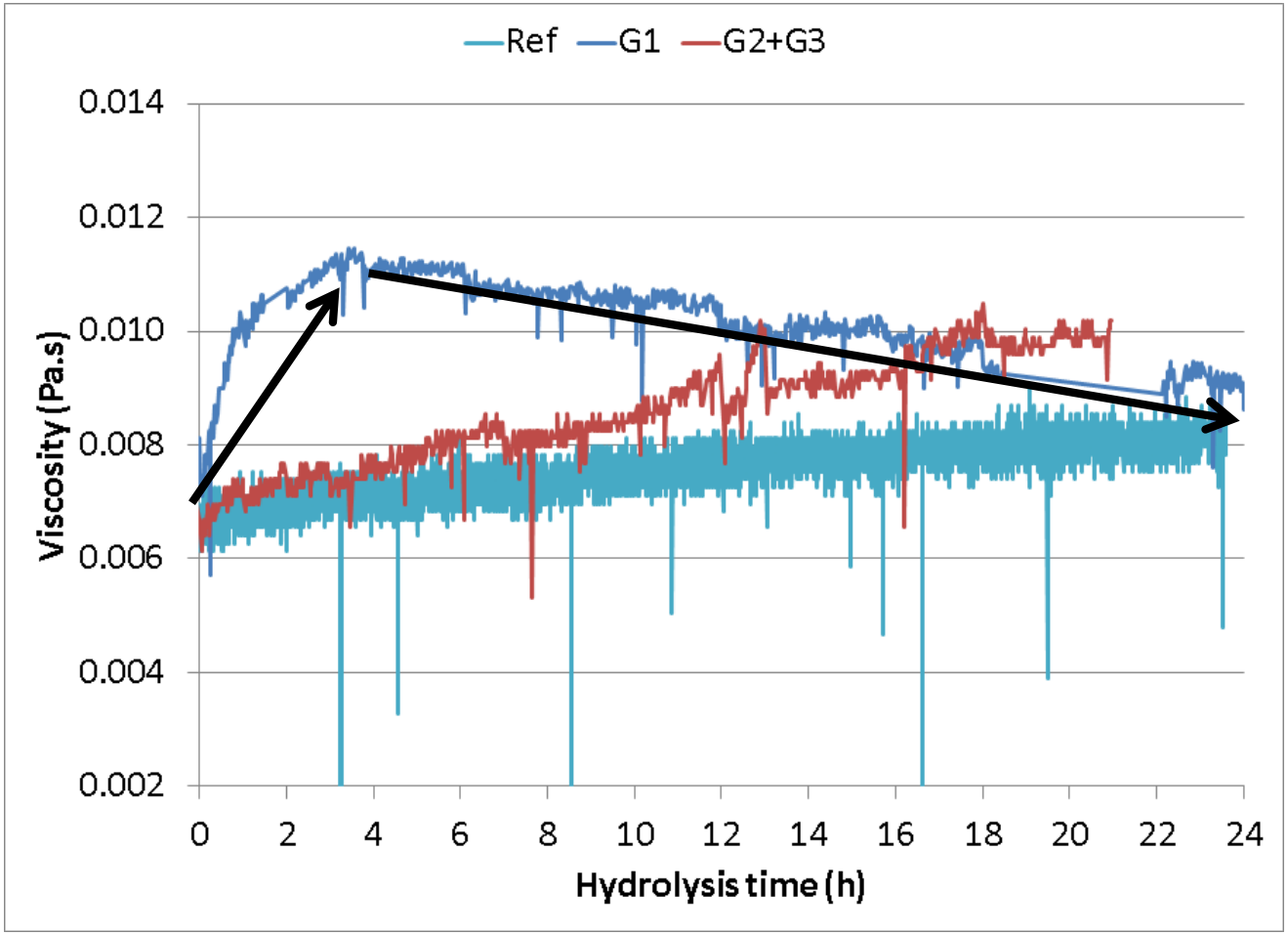
1. G2+G3 = no effect, G1 = predominant
2. Synergy observed on G1+G2+G3.
3. Ctec2 = stronger liquefaction efficiency. It is likely explained by higher activities content of exo-glucanase and β-glucosidase.

Results on sugarcane bagasse



Sugarcane bagasse 30gdm/L
Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **gradual rise in viscosity.**

Results on sugarcane bagasse

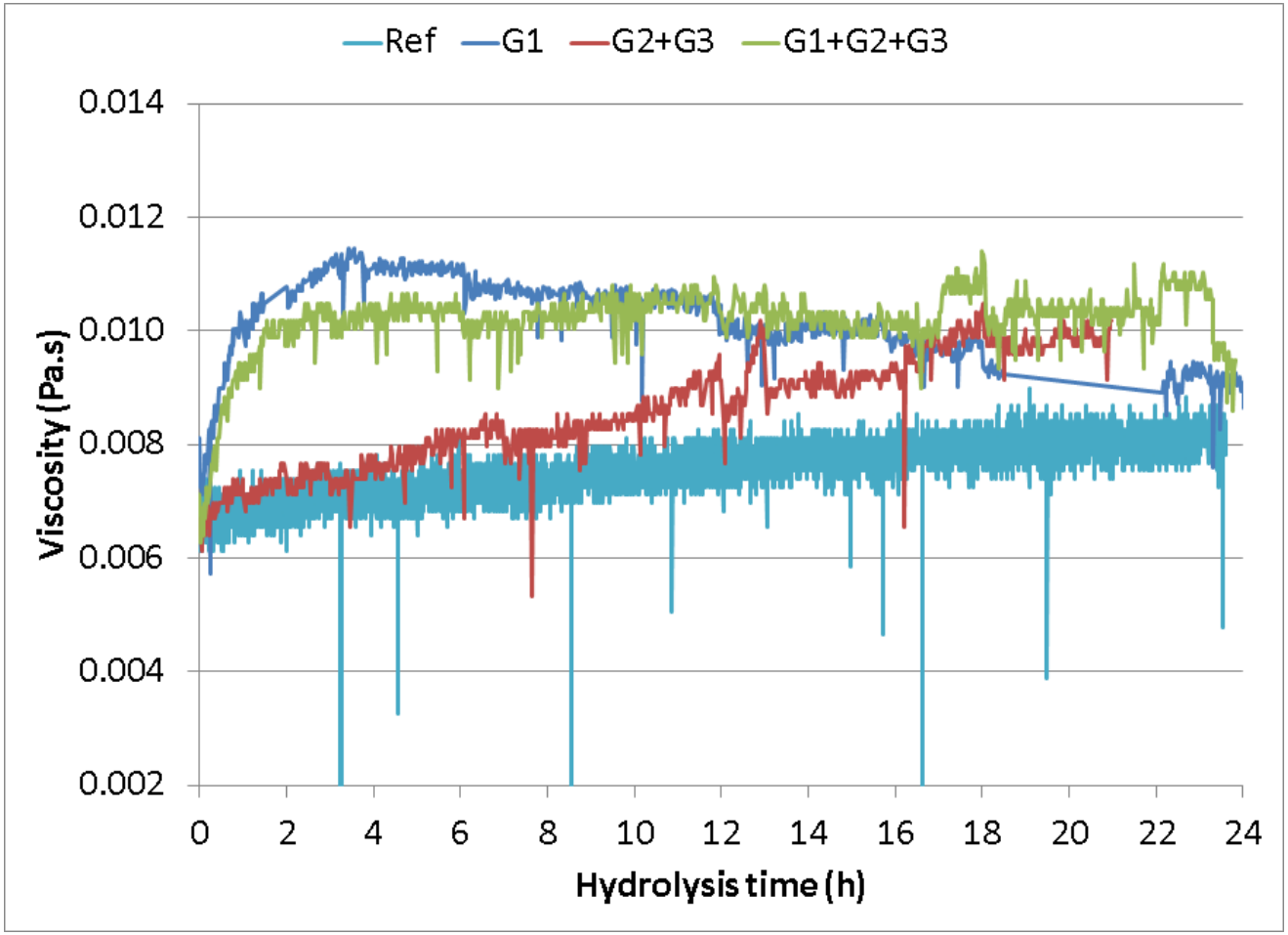


Sugarcane bagasse 30gdm/L
Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **small contribution.**

G1 (endo-glucanase) = **rise** of suspension viscosity in the first 3h.

RESULTS & DISCUSSION

Results on sugarcane bagasse

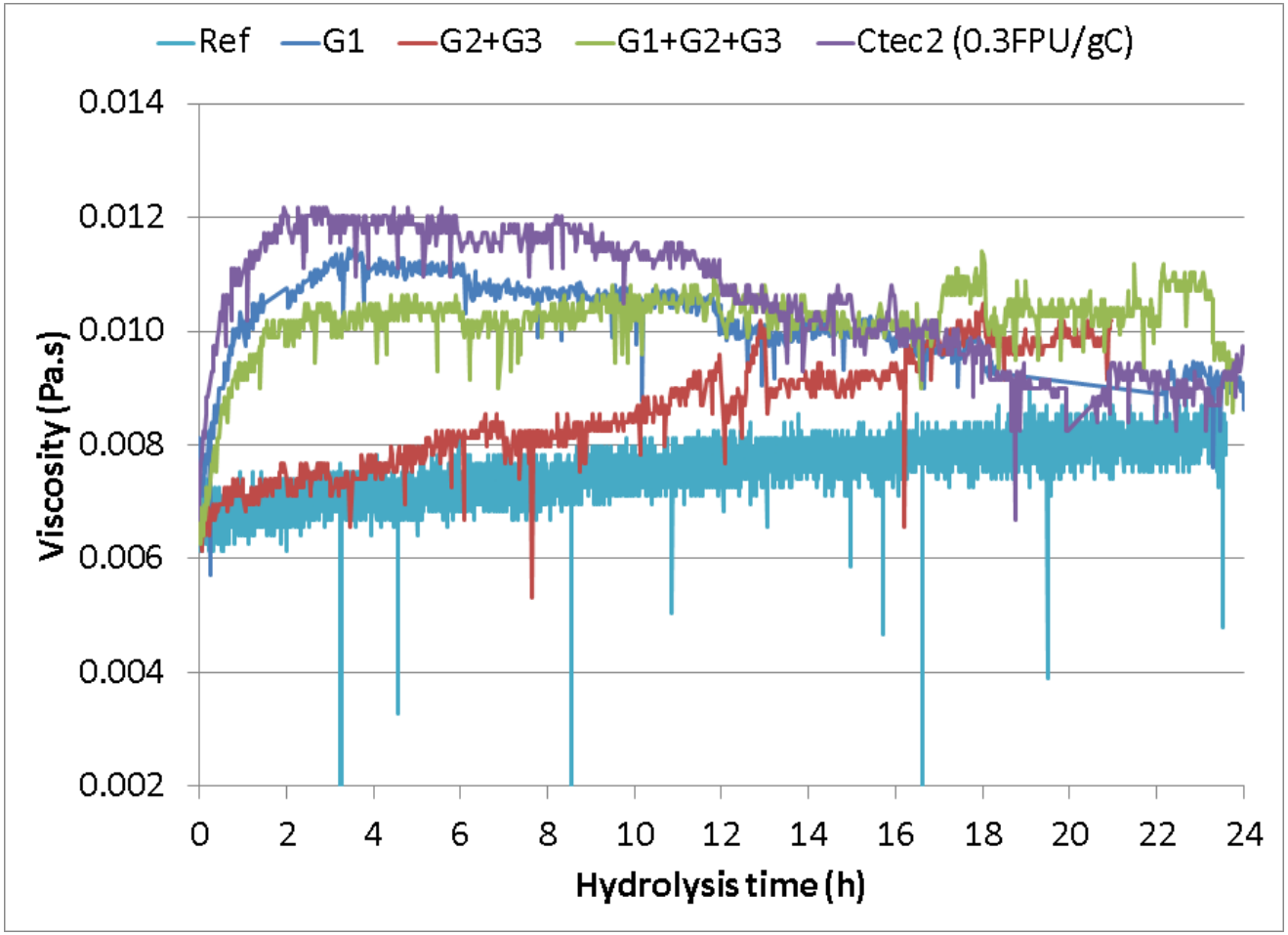


Sugarcane bagasse 30gdm/L
Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **small contribution.**

G1 (endo-glucanase) = **rise** of suspension viscosity in the first 3h.

G1+G2+G3 similar viscosity pattern with either **G1** alone or **Ctec2**.

Results on sugarcane bagasse



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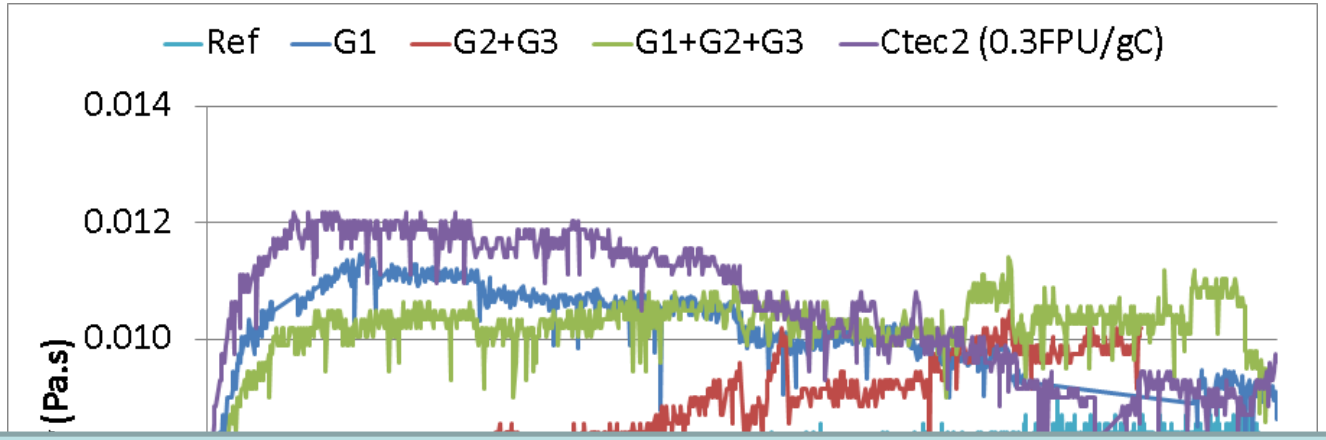
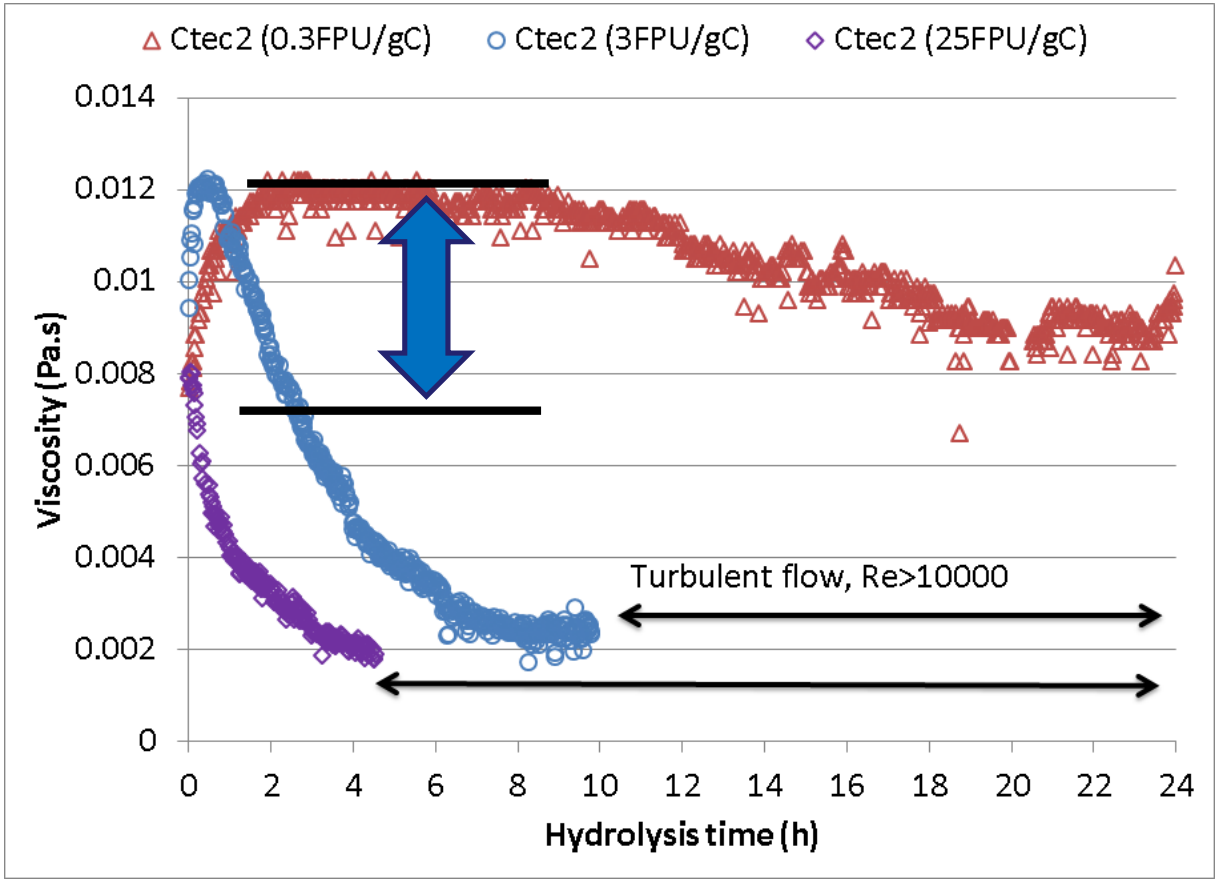


Sugarcane bagasse 30gdm/L
Mixing rate 100rpm
Ref: no enzyme
G2+G3 (exo-glucanase & β-glucosidase) = **small contribution.**

Message 2:

- Expected results :
  - G1=predominant role, G2+G3=minor contribution.
  - Synergy between G1, G2 and G3 ➔ liquefaction efficiency.
- **Original insight:**
  - Swelling step of sugarcane bagasse. Mechanism???

Swelling of SCB suspension

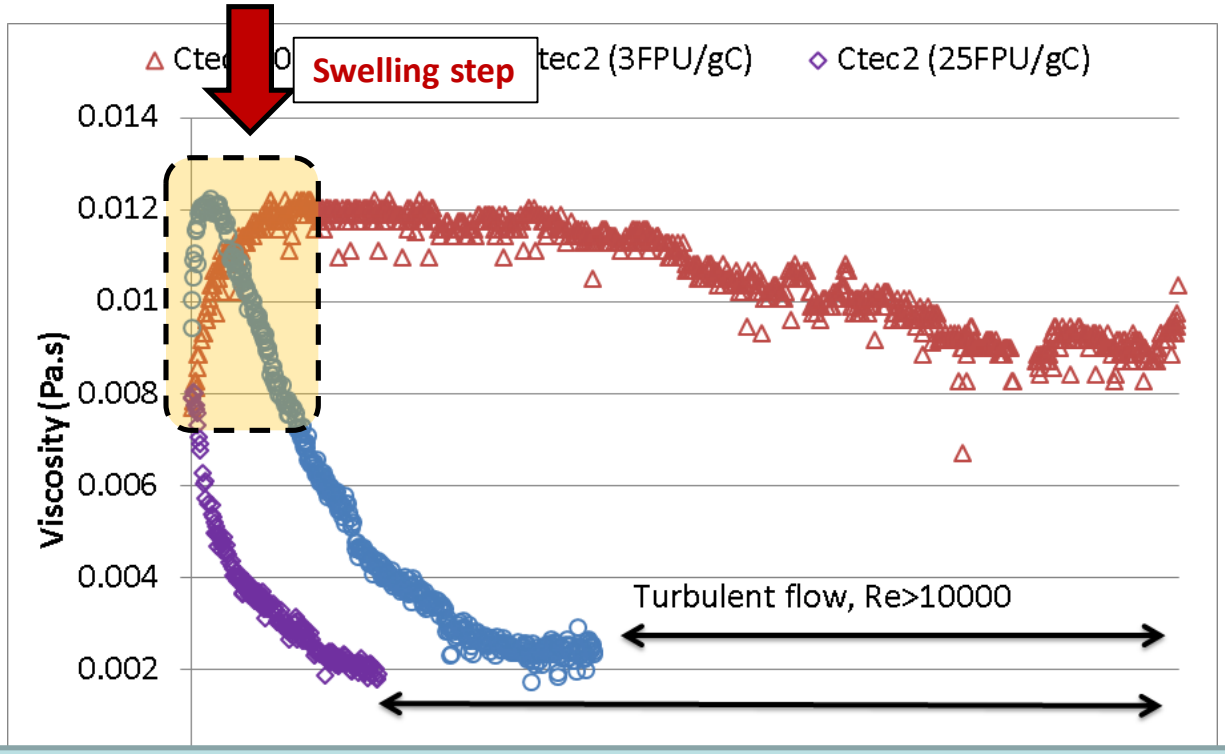


SCB 30gdm/L
Mixing rate 100rpm

No rise in viscosity observed at high enzyme loading (25FPU/gC)

Same level of increase in viscosity with 0.3 & 3 FPU/gC

Swelling of SCB suspension



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Mixing rate 100rpm

No rise in viscosity observed at high enzyme loading (25FPU/gC)

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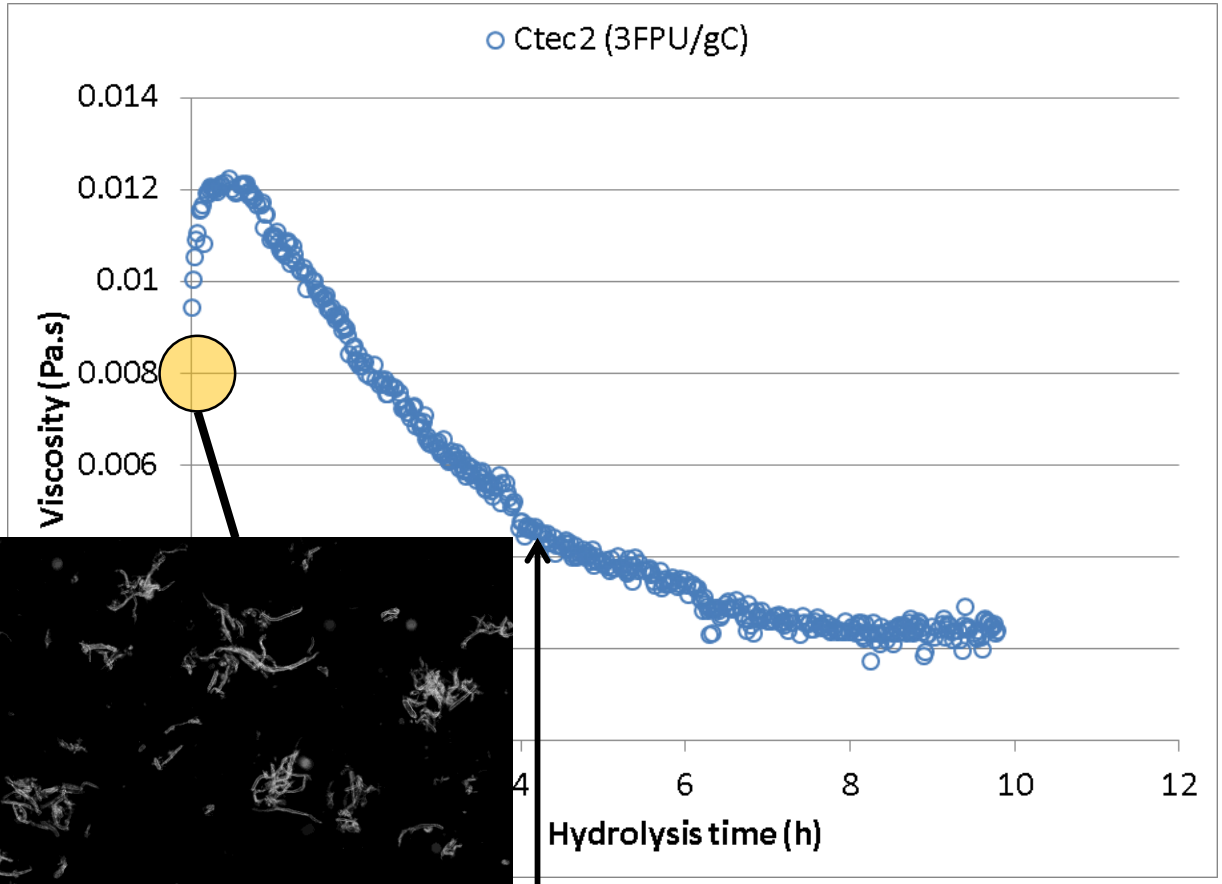
From literature:

H1: ↑[s] lead to ↑viscosity (Fan et al., 2003; T.C. Nguyen et al. 2013, this work)

H2: ↑particle size lead to ↑viscosity (Dasari and Berson 2007) → **Need to be verified**

Swelling of SCB suspension



0.3cm x 0.3cm

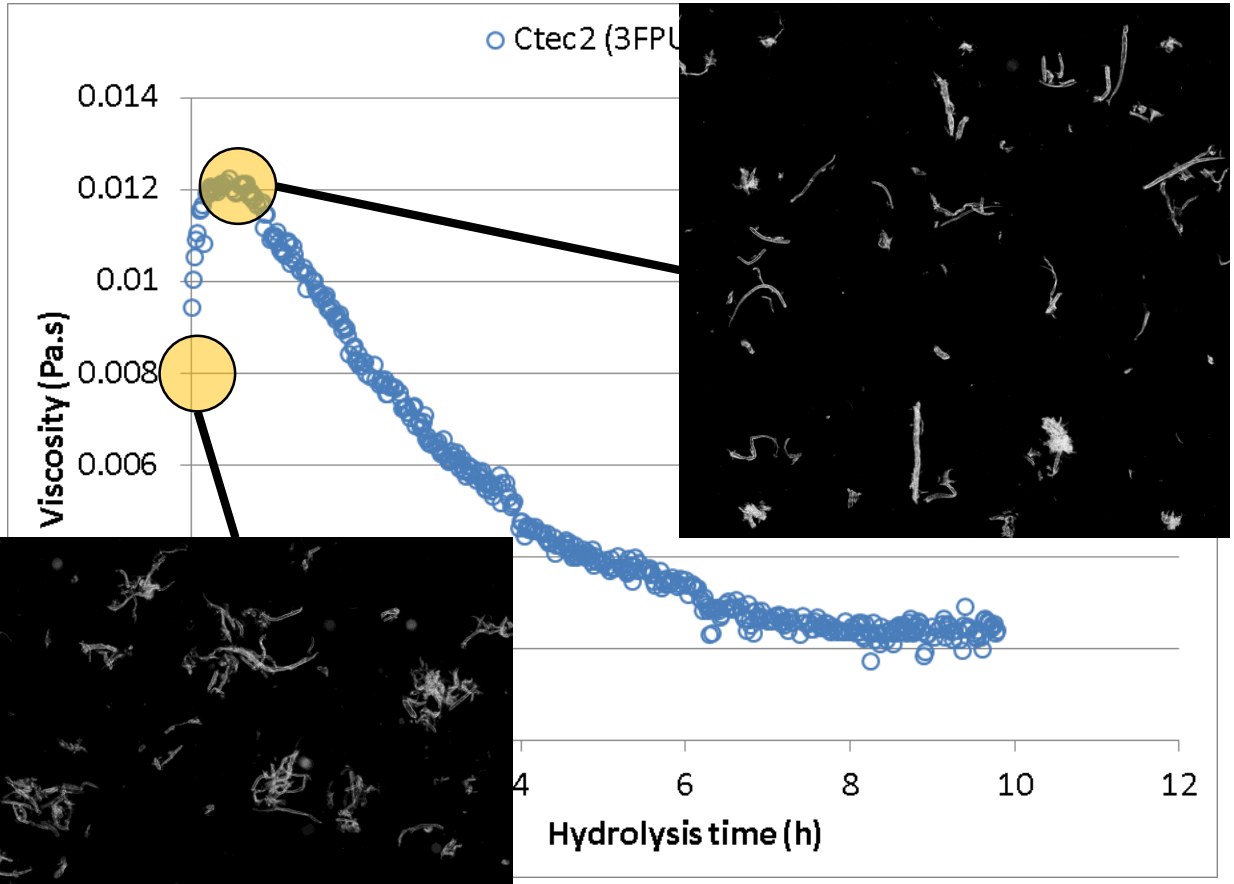
Observation mode: top light, dilution 20, area 1x1cm².

Data from Morphology

<u>At 0h:</u>

- Considerable number of agglomerates.
- Mean dCE=**12.98**±1.34µm

Swelling of SCB suspension



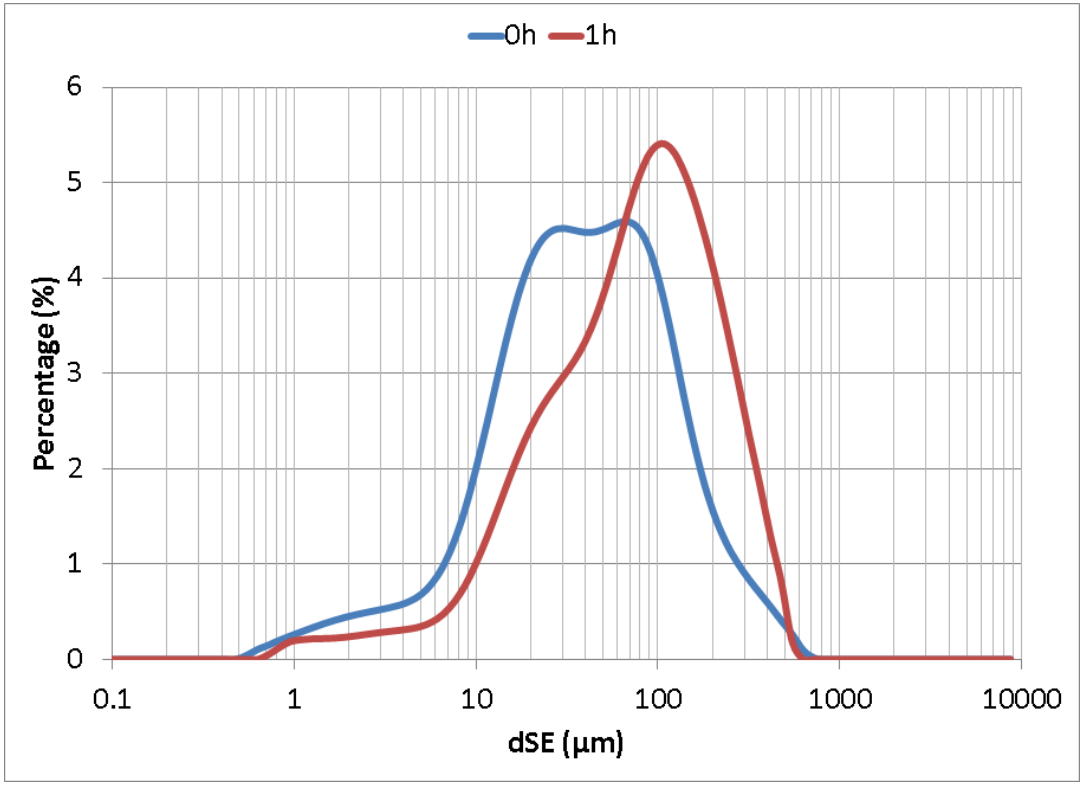
Data from Morphology

<u>At 0h:</u>
- Considerable number of agglomerates.
- Mean dCE=**12.98**±1.34µm

<u>At 1h:</u>
- Few agglomerates, several individuals particles.
- Mean dCE=**18.76** µm

Increase in mean dCE
Disappear of agglomerates

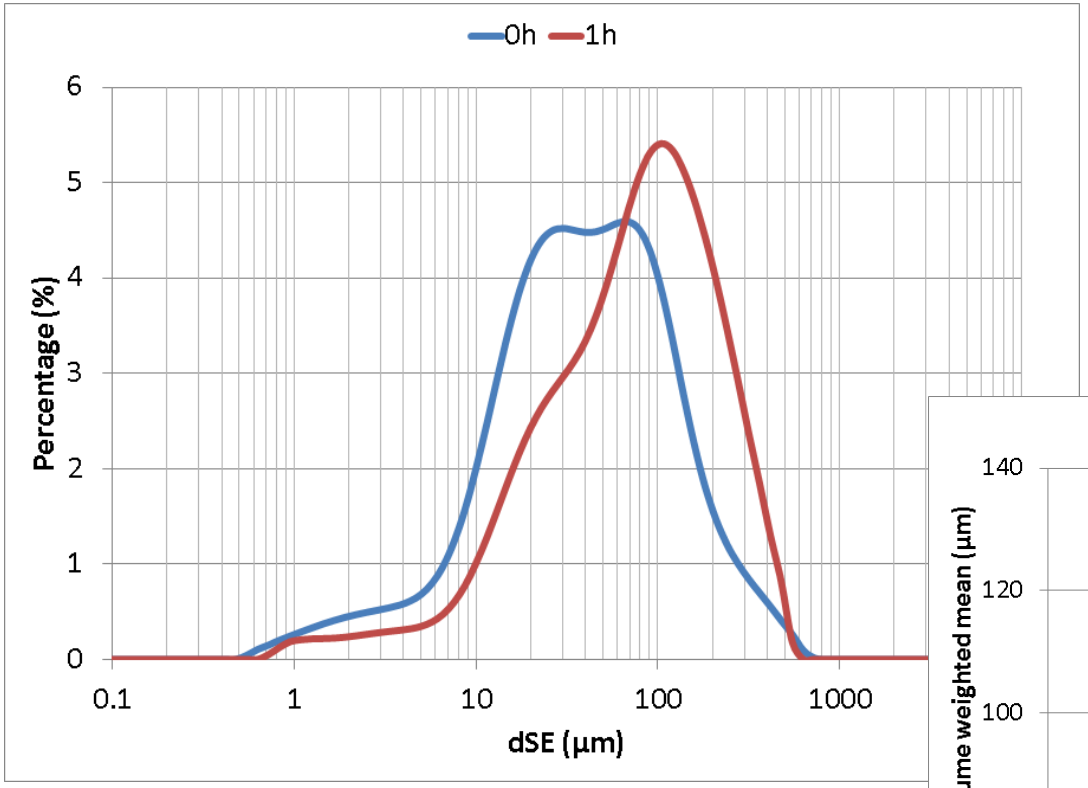
Swelling of SCB suspension



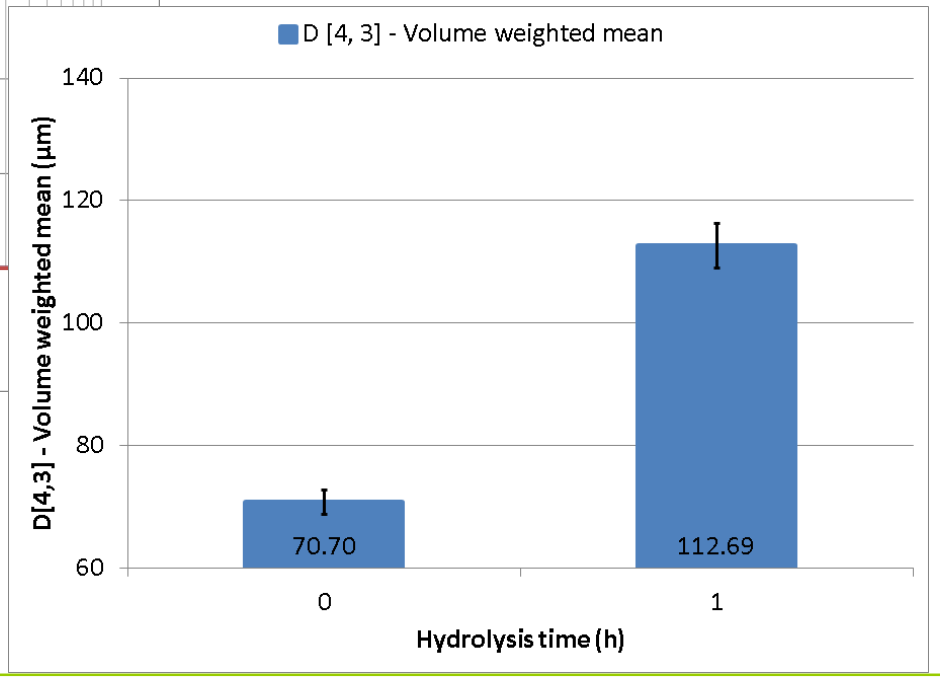
Data from DLS

dSE = diameter of sphere equivalent.

Swelling of SCB suspension

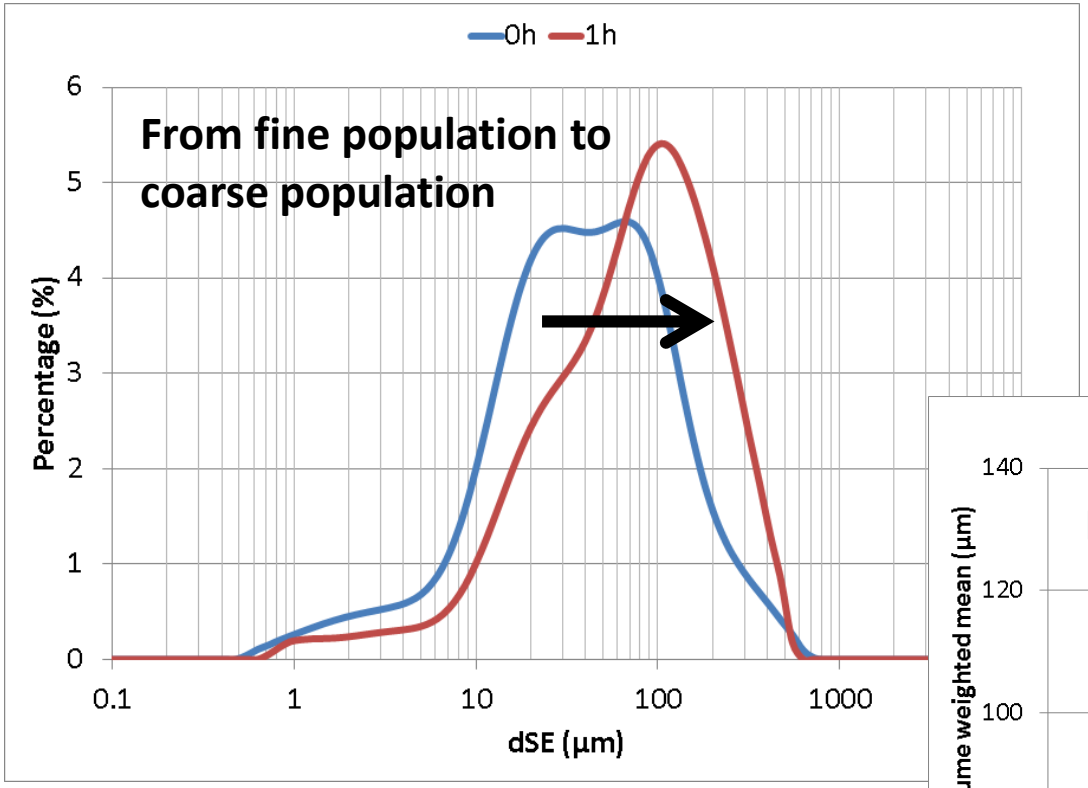


Data from DLS



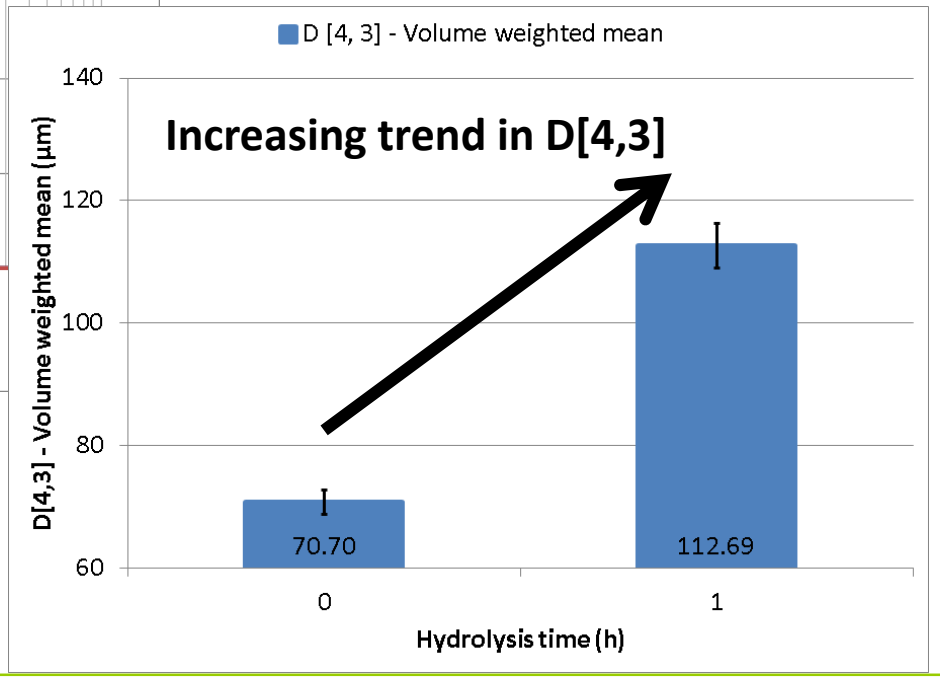
dSE = diameter of sphere equivalent.

Swelling of SCB suspension



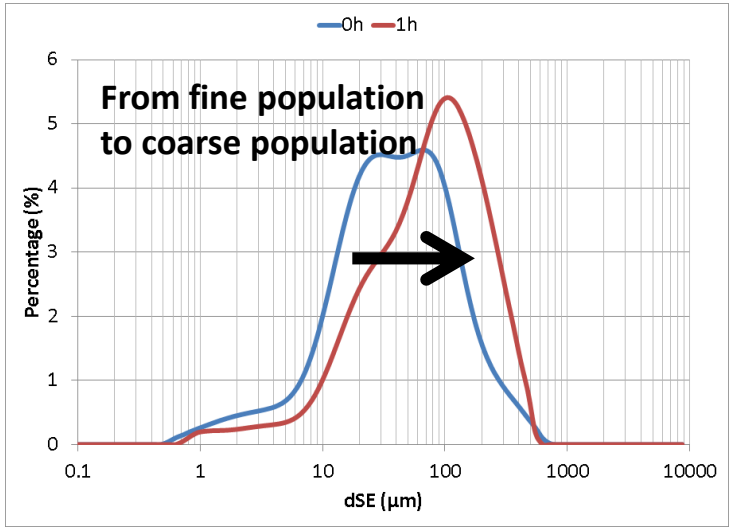
Data from DLS

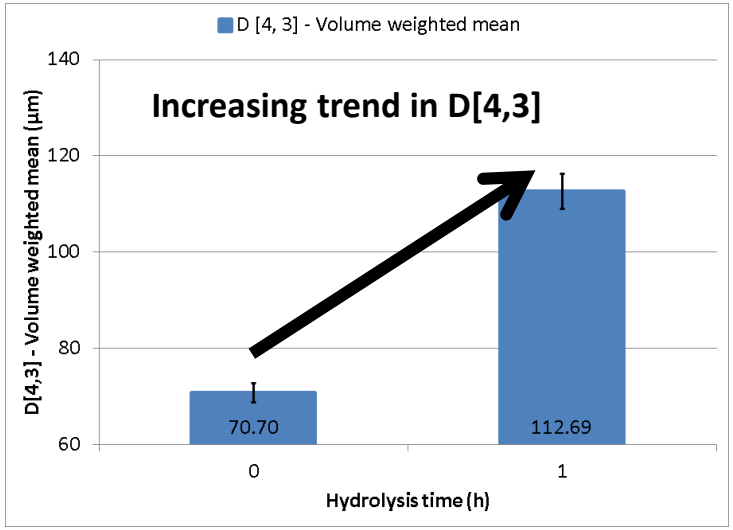
Solubilization of fine population.



dSE = diameter of sphere equivalent.

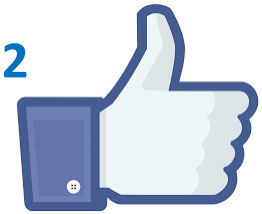
Swelling of SCB suspension





Phenomenon observed: rise in viscosity

Hypothesis 2

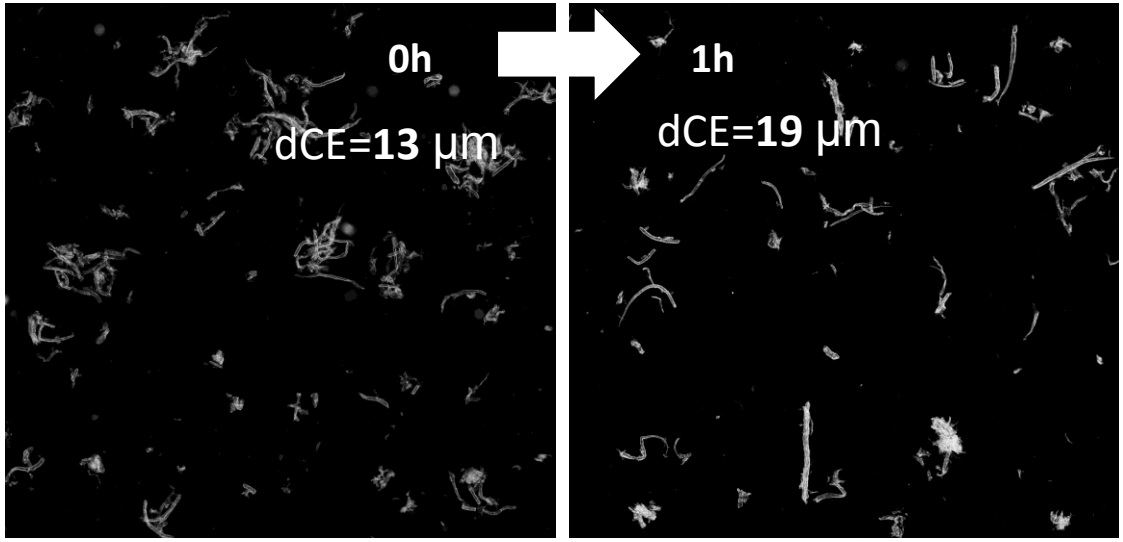


Analysis results

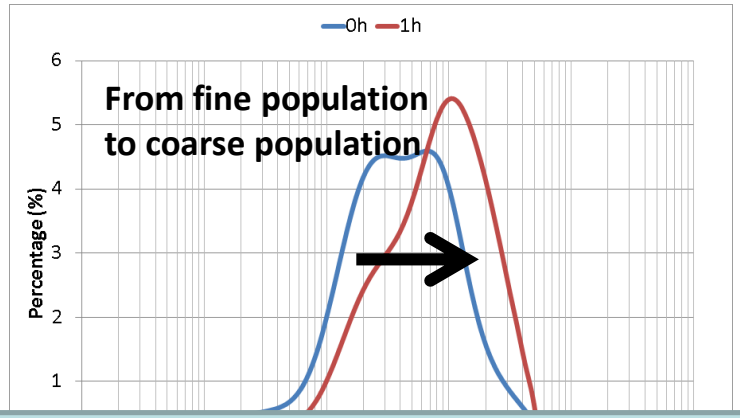
Cellulose to glucose conversion **3.45±0.45%**

➔ Negligible variation of **[S]**

DLS + Morpho: increase in particle size



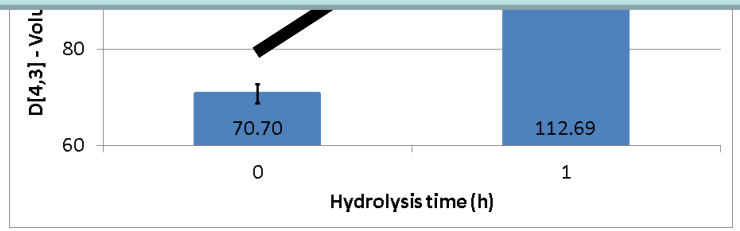
Swelling of SCB suspension

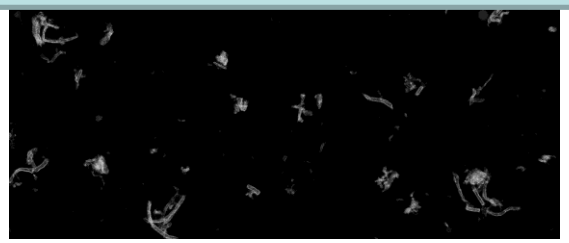


- **Solubilization of fine population.**
- **Increase in particle mean diameter**
- **Particles were separated from agglomerates**

Message 3

- **Swelling step on SCB suspension = evolution of population from fine to coarse**
- **Obtained results are not enough to reveal its mechanism.**

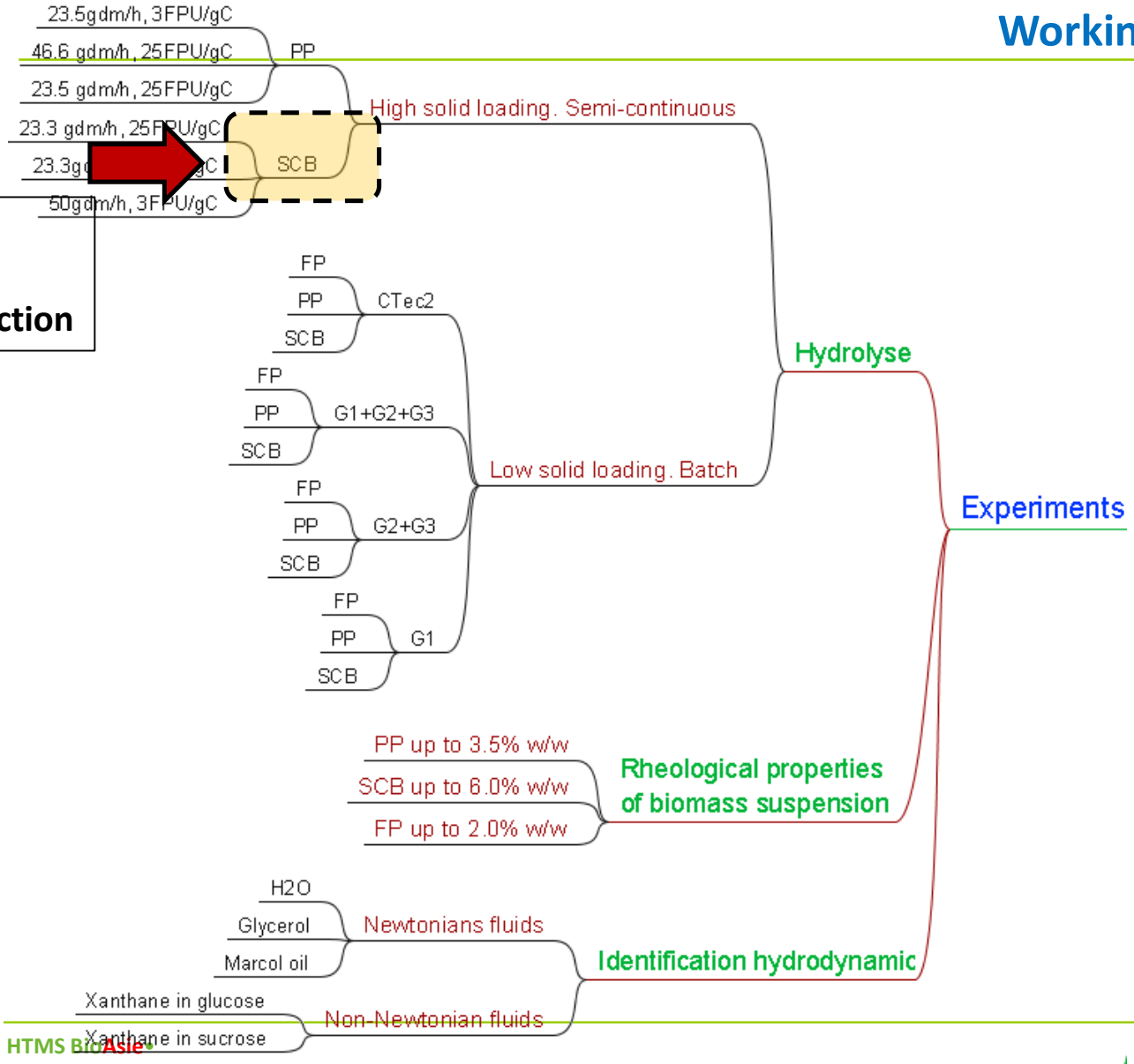




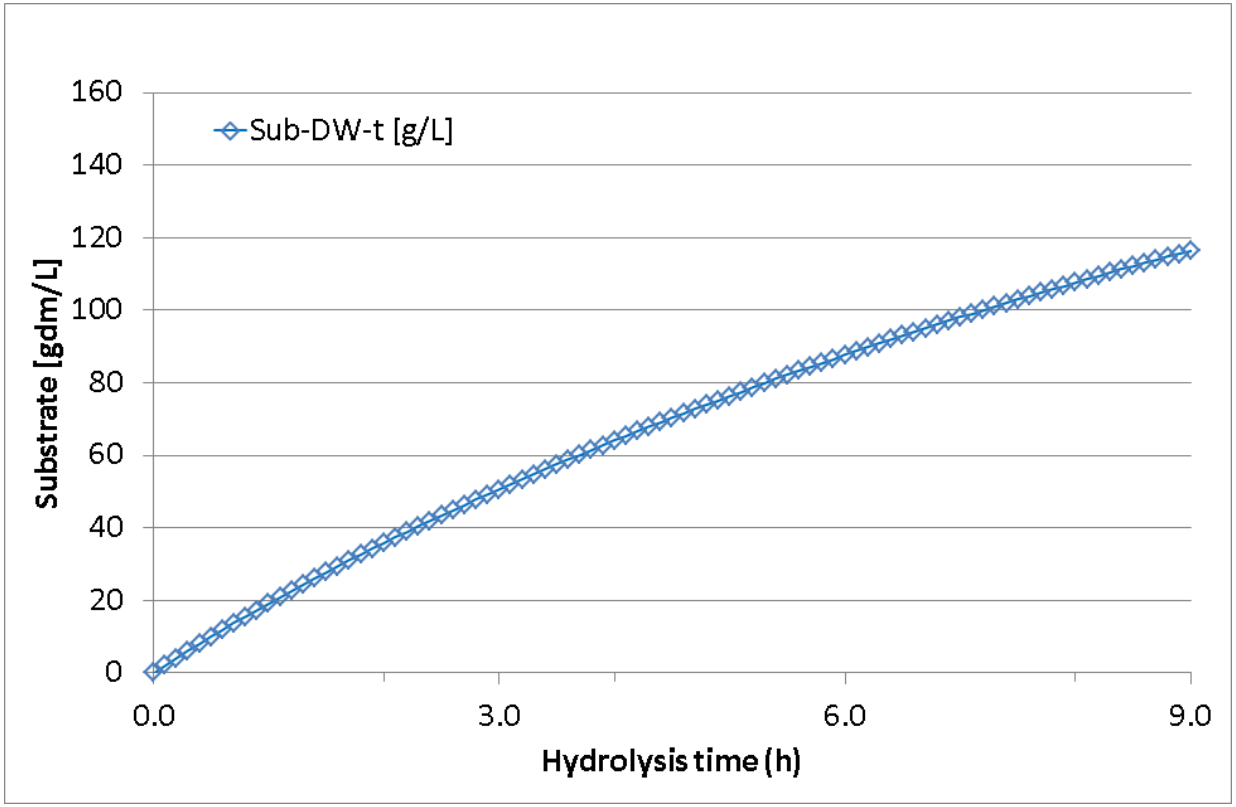
Research STRATEGY

Working diagram

Message 4:
Mechanism of enzymatic liquefaction

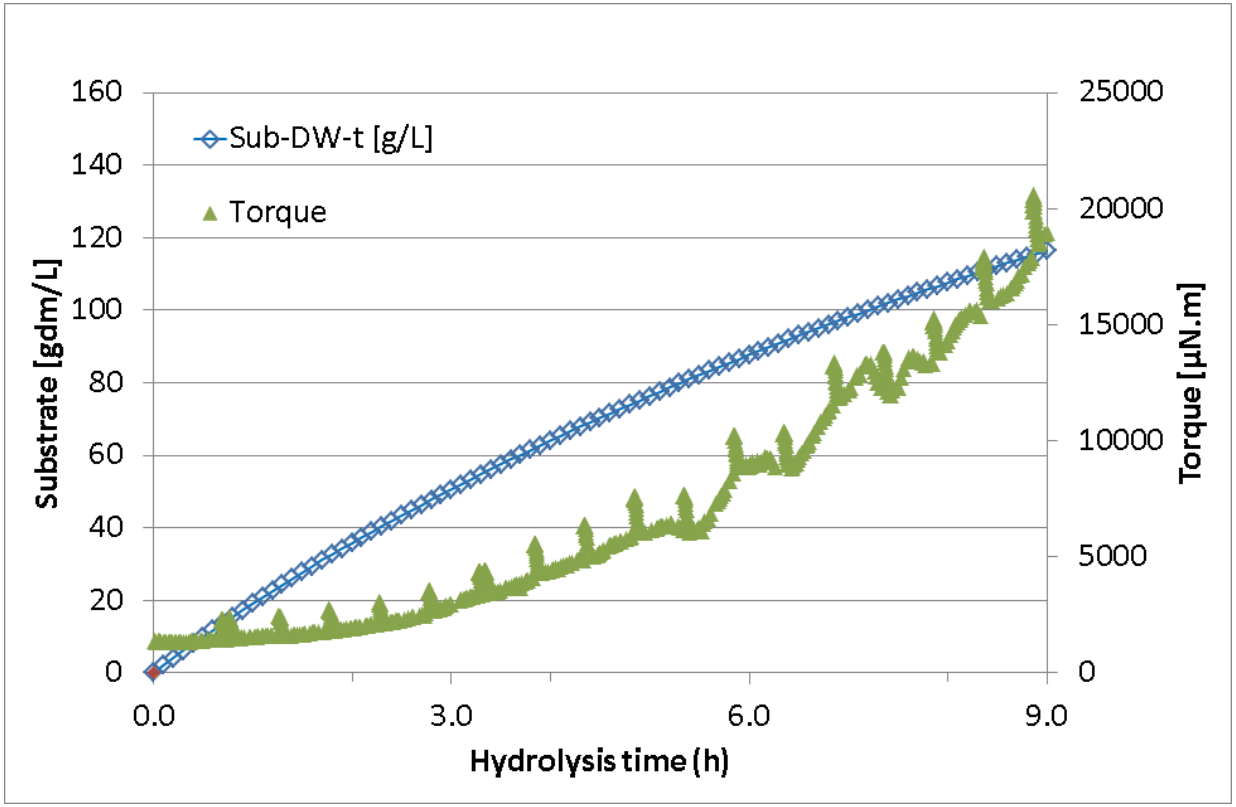


Experiment overview: from raw to interpreted data



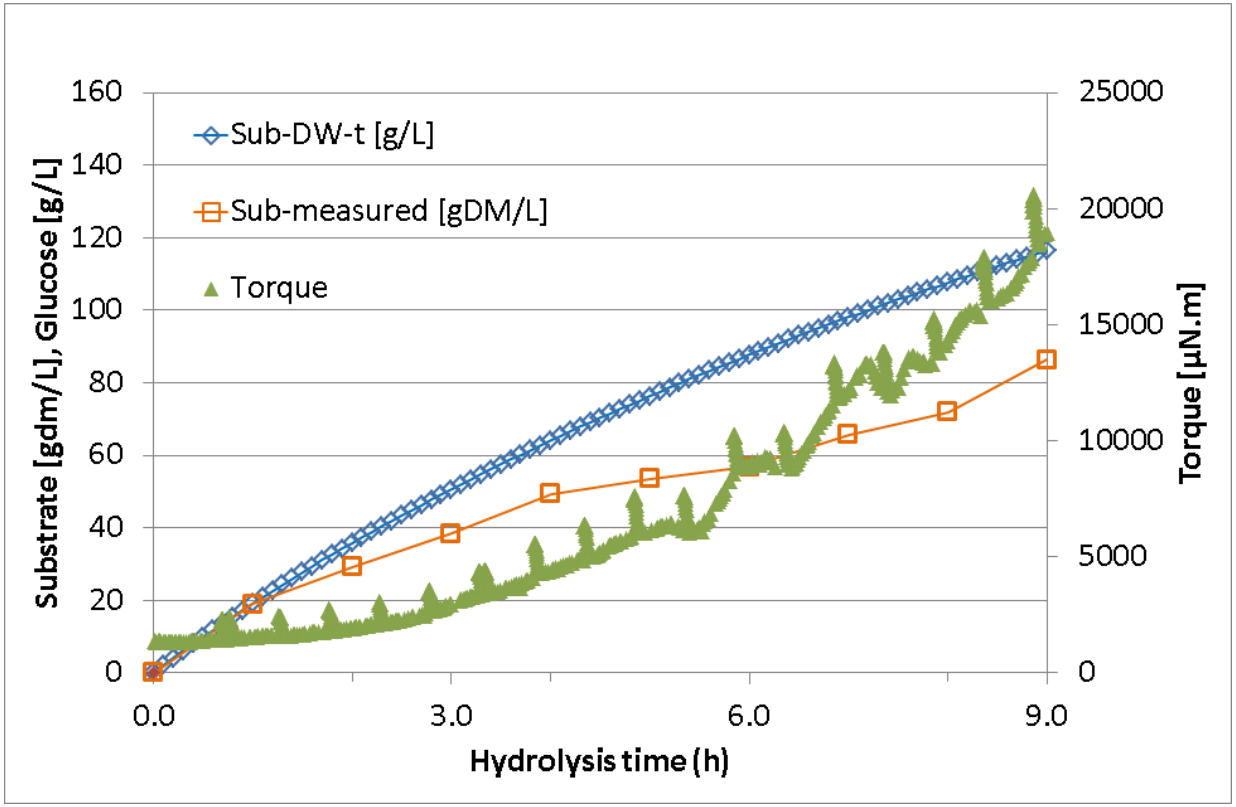
- **Substrate** was feed at constant rate Qs (g/h)
- **Enzyme** was feed with substrate at fixed ratio Qe/Qs=const

Experiment overview: from raw to interpreted data



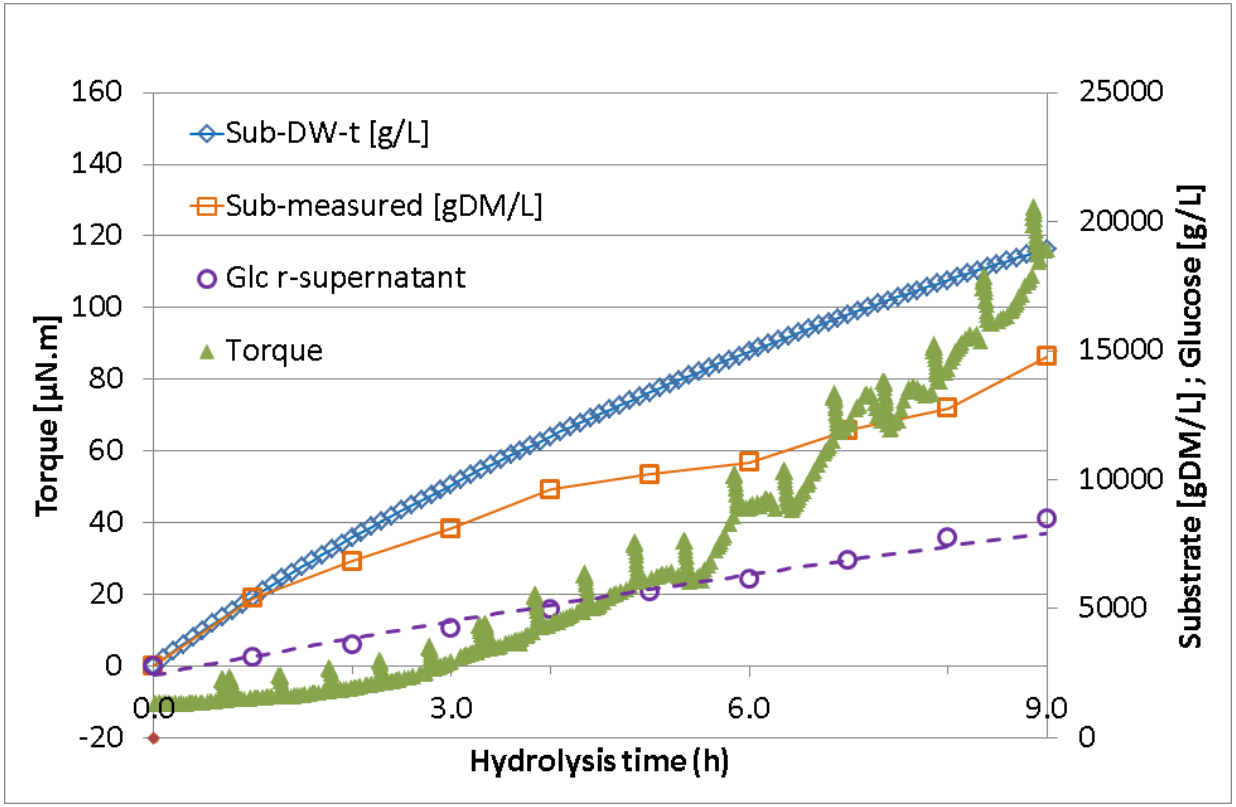
- **Substrate** was feed at constant rate Qs (g/h)
- **Enzyme** was feed with substrate at fixed ratio Qe/Qs=const
- **Torque** values were recorded every 1min

Experiment overview: from raw to interpreted data



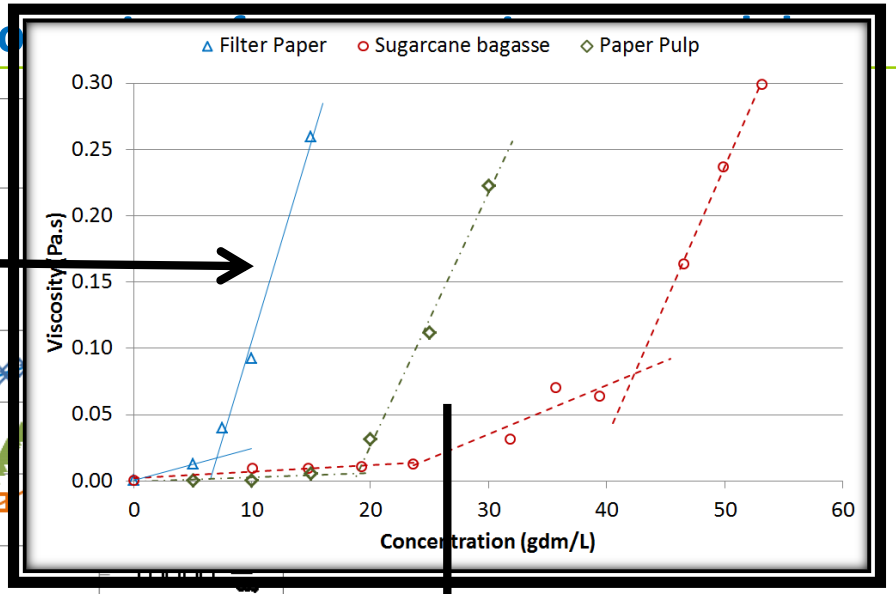
- **Substrate** was feed at constant rate Qs (g/h)
- **Enzyme** was feed with substrate at fixed ratio Qe/Qs=const
- **Torque** values were recorded every 1min
- Periodically, samples were taken for analysis of **dry matter content**

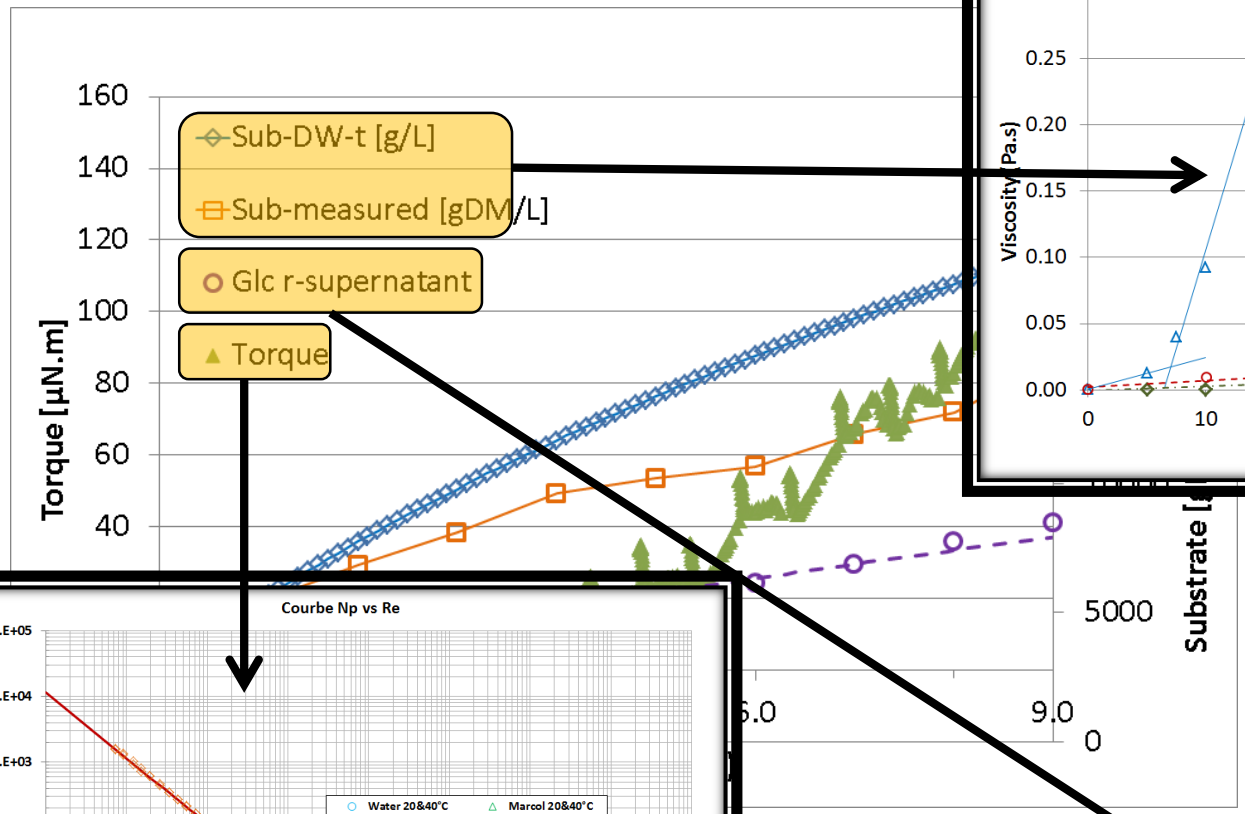
Experiment overview: from raw to interpreted data

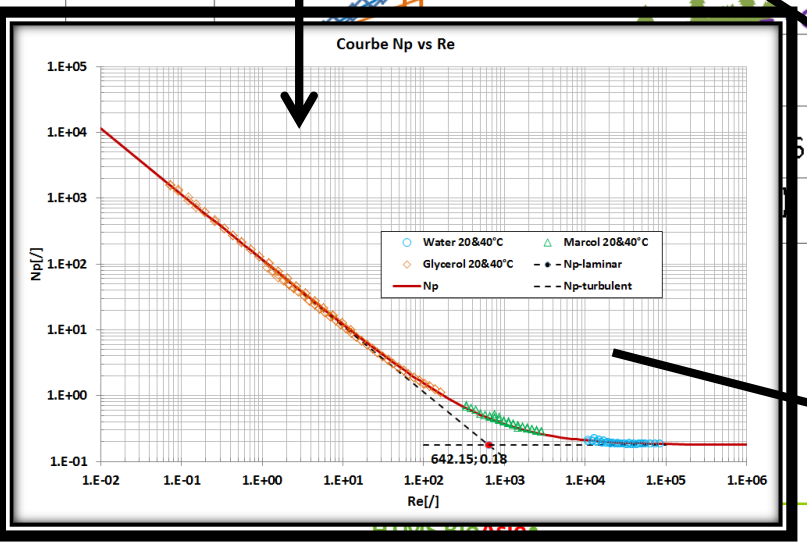


- **Substrate** was feed at constant rate Qs (g/h)
- **Enzyme** was feed with substrate at fixed ratio Qe/Qs=const
- **Torque** values were recorded every 1min
- Periodically, samples were taken for analysis of **dry matter content** and **glucose**

Experiment c





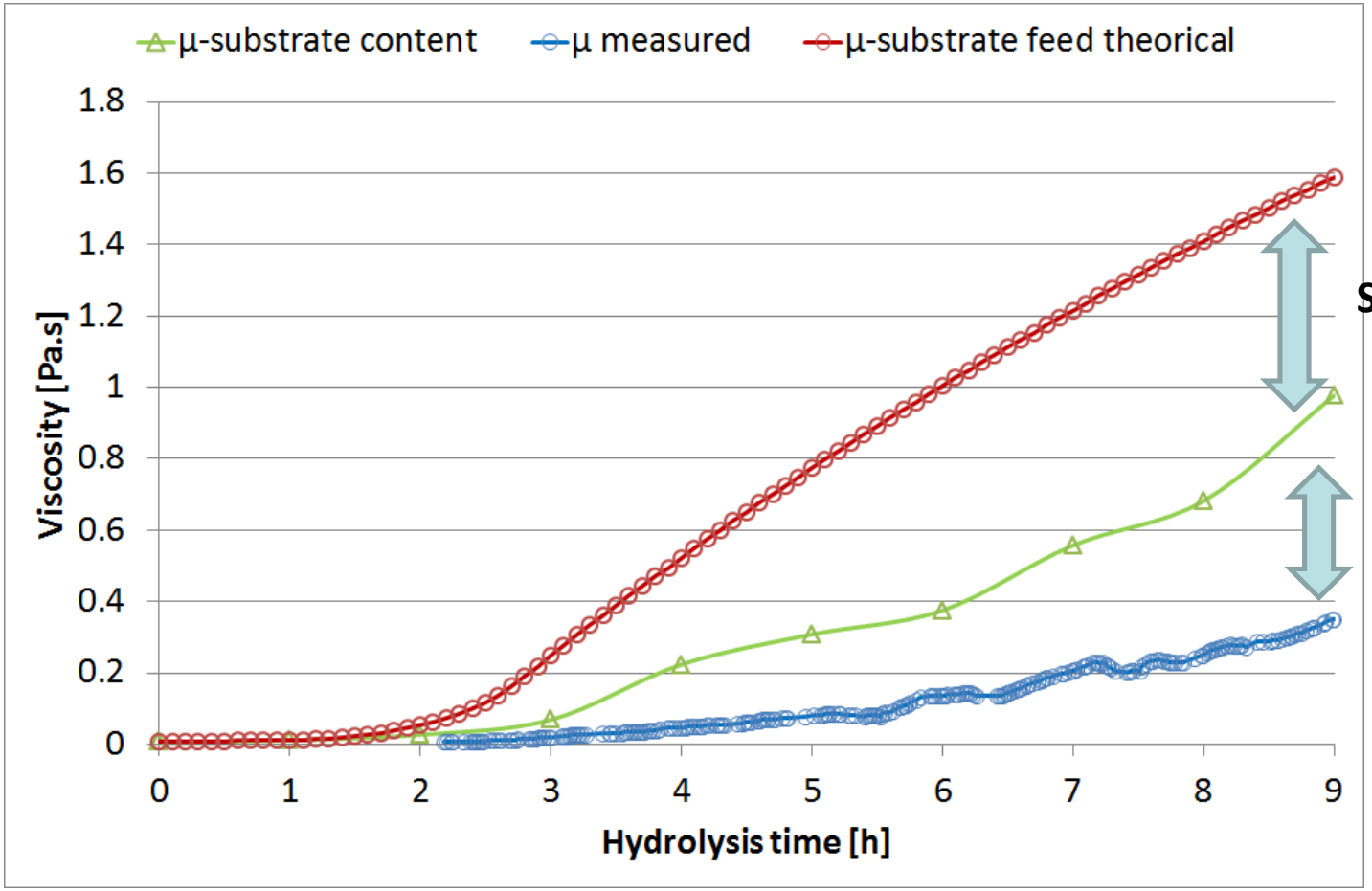


Theoretical viscosity

Hydrolysis **yield, kinetic**

In-situ **viscosity**

Mechanism of liquefaction

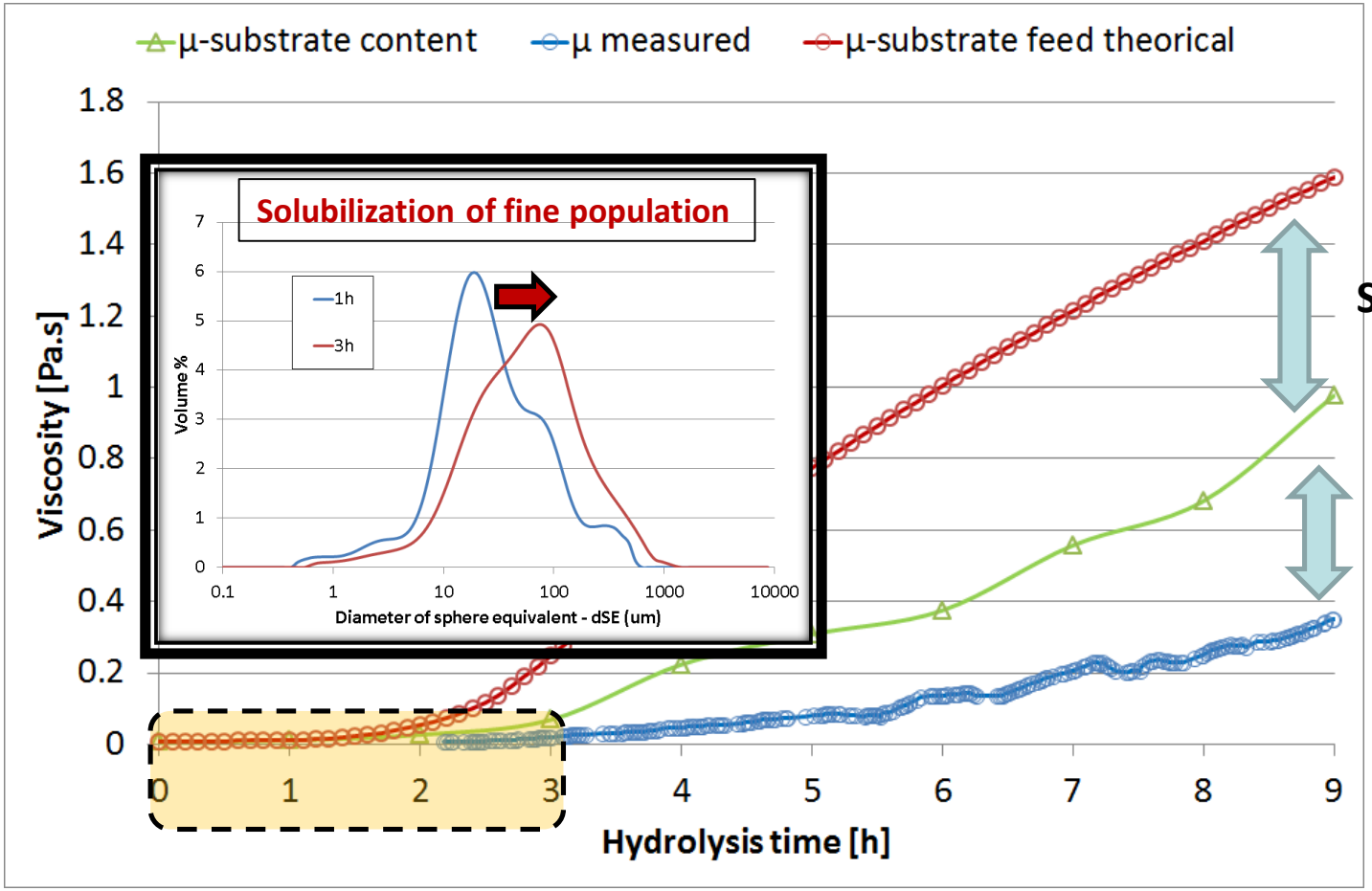


Final [SCB] **140** gdm/L
Qs = **23.3** gdm/h
Enzyme: **25**FPU/g cellulose
Phase feeding

Substrate solubilization

Fiber modification

Mechanism of liquefaction



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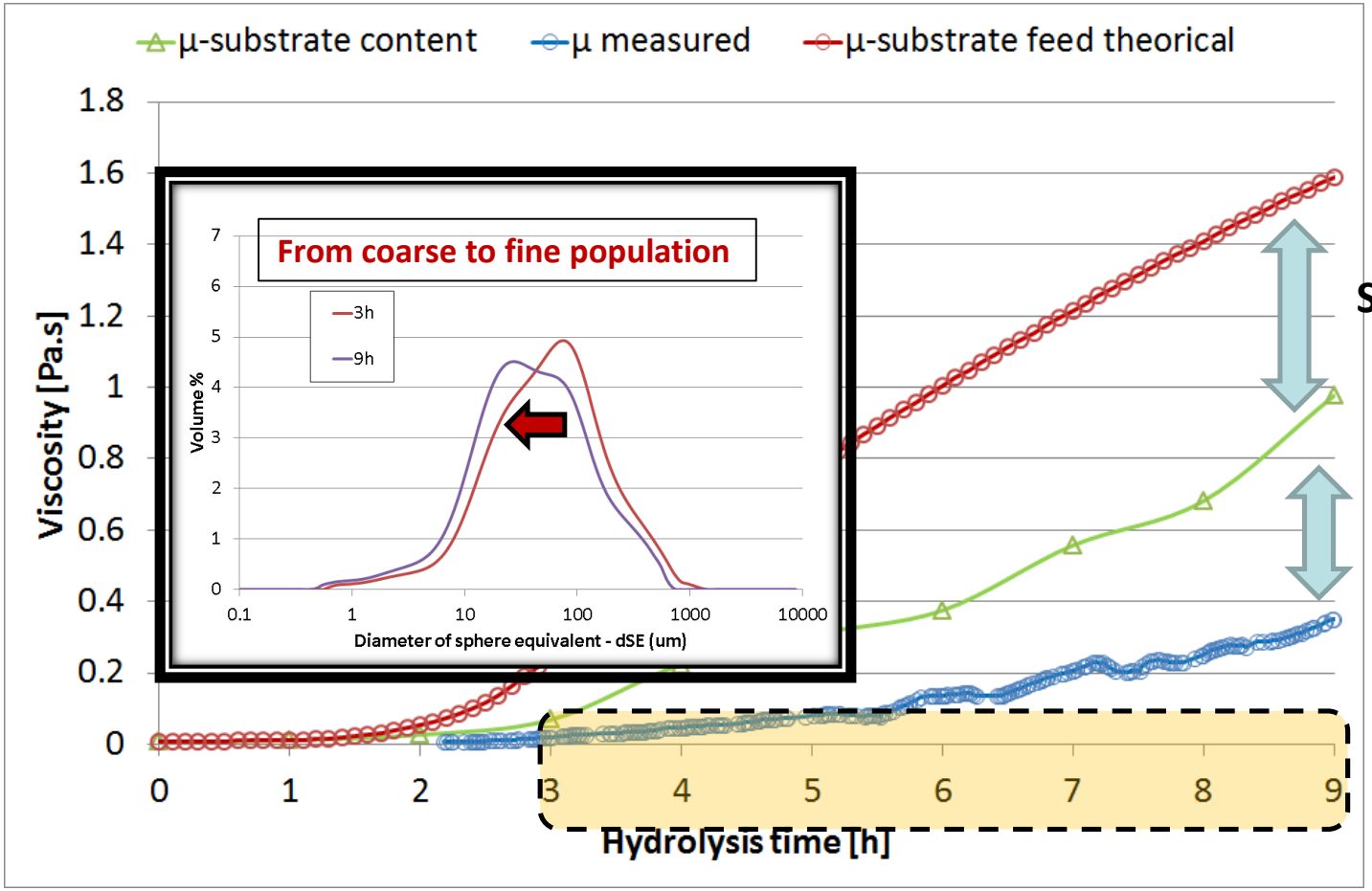

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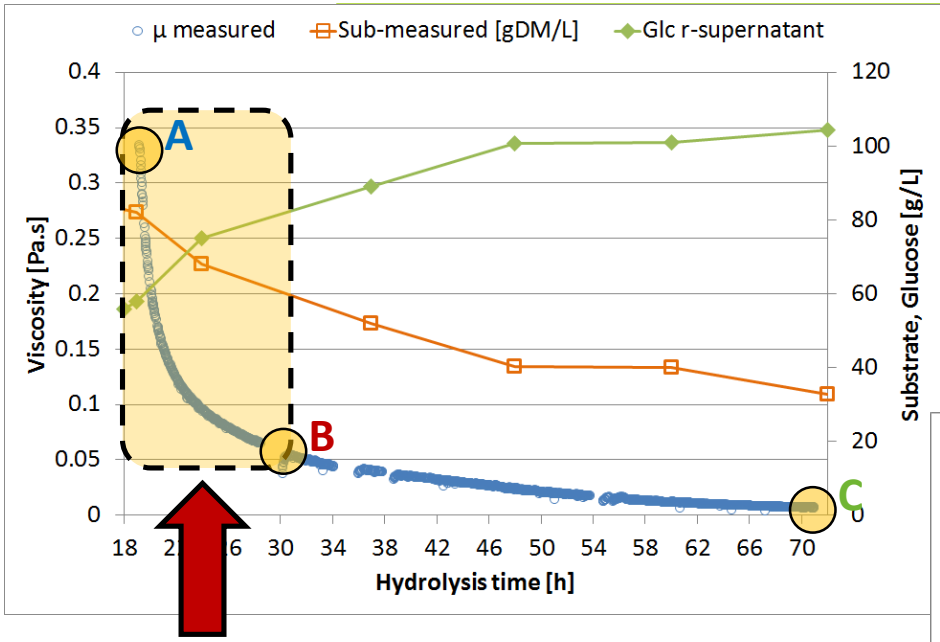
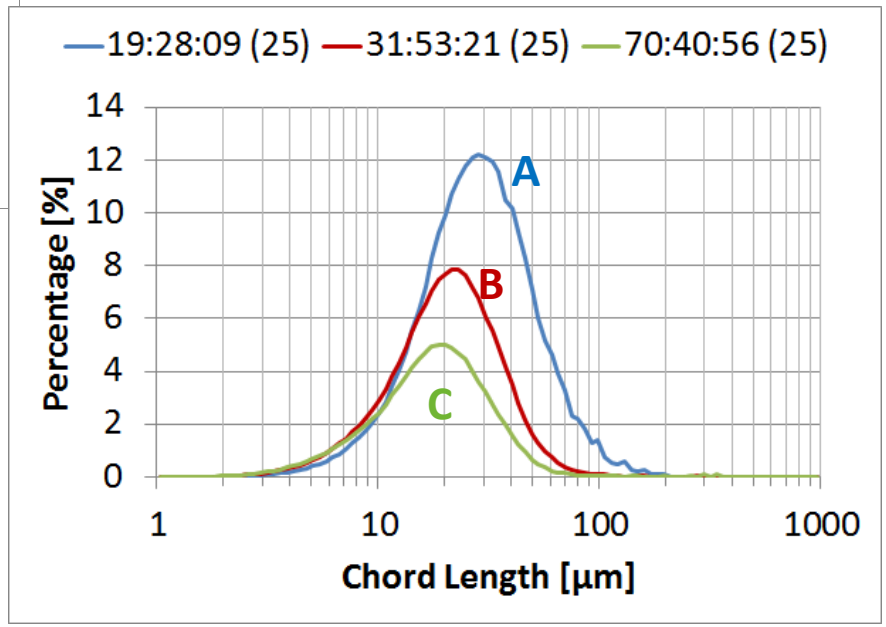


Drastic fall in viscosity

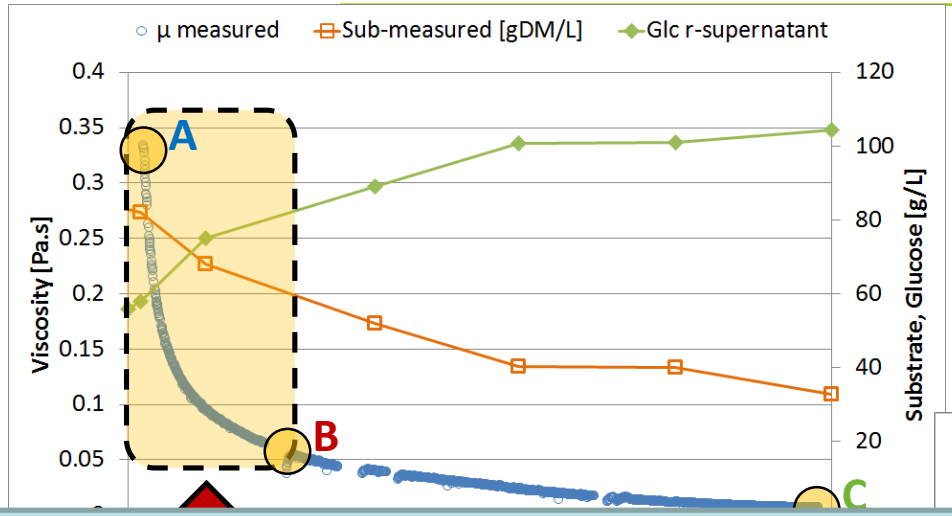
From **A** to **B**: big particles were broken down into smaller particles.

From **B** to **C**: solubilization of small particles

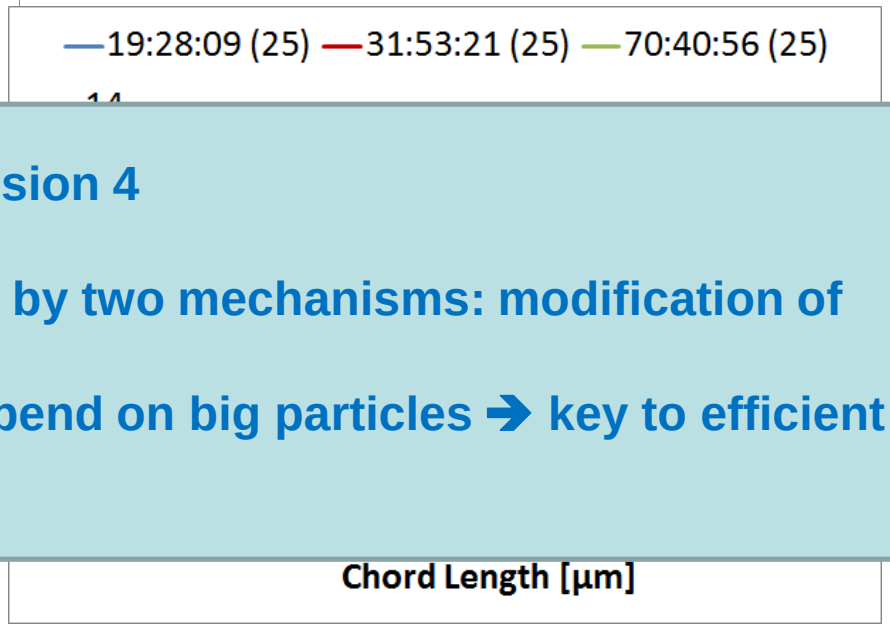
End of feeding
Final [SCB] = **140** gdm/L
Enzyme: **25**FPU/g cellulose



Mechanism of liquefaction



End of feeding
Final [SCB] = **140** gdm/L
Enzyme: **25**FPU/g cellulose

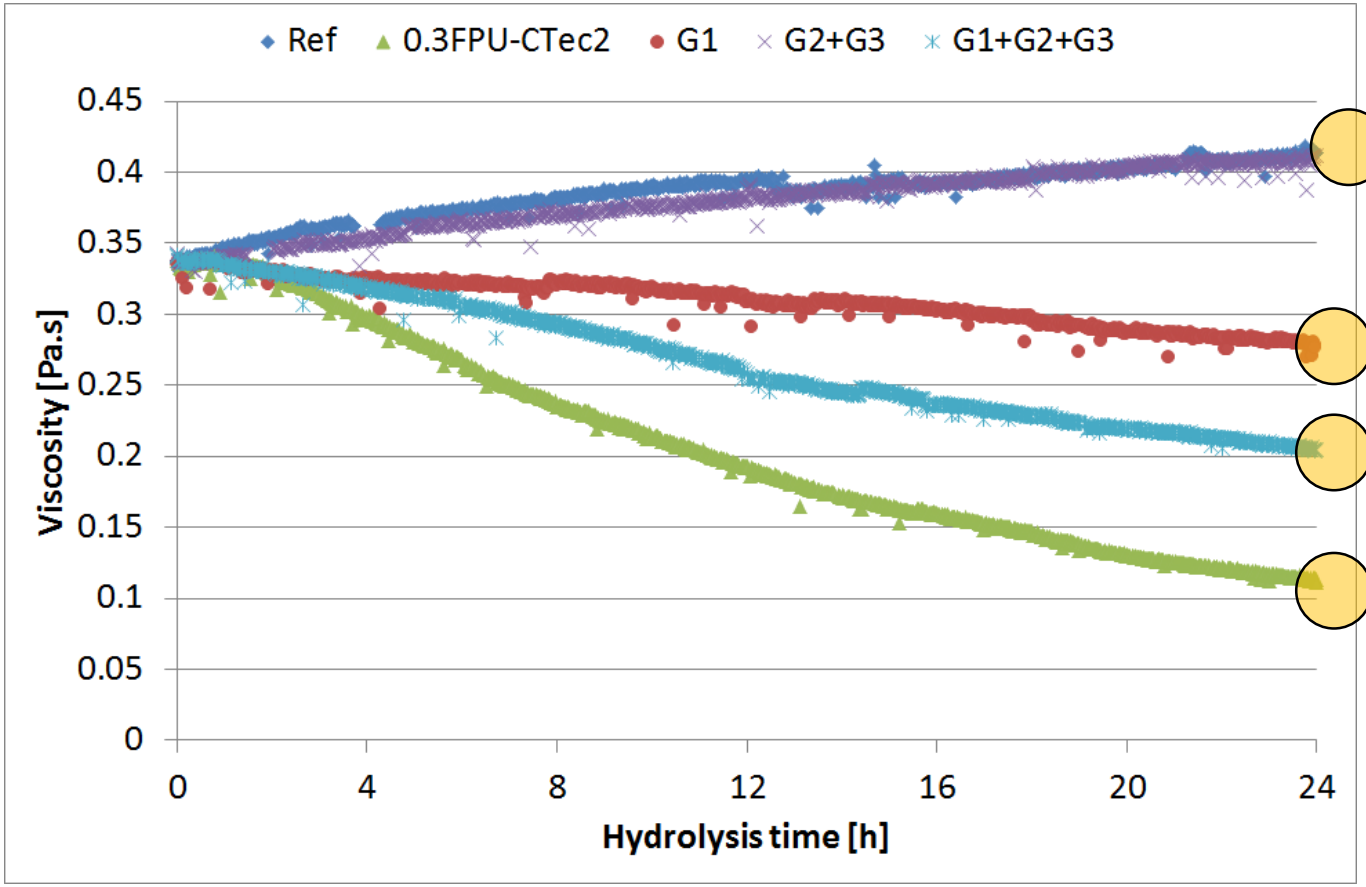


Conclusion 4

- **Enzyme reduces suspension viscosity by two mechanisms: modification of fiber and solubilization of substrate.**
- **Suspension viscosity was strongly depend on big particles ➔ key to efficient liquefaction.**

Relationship between physical and biochemical results

Paper pulp 30gdm/L



Glc **0g/L**

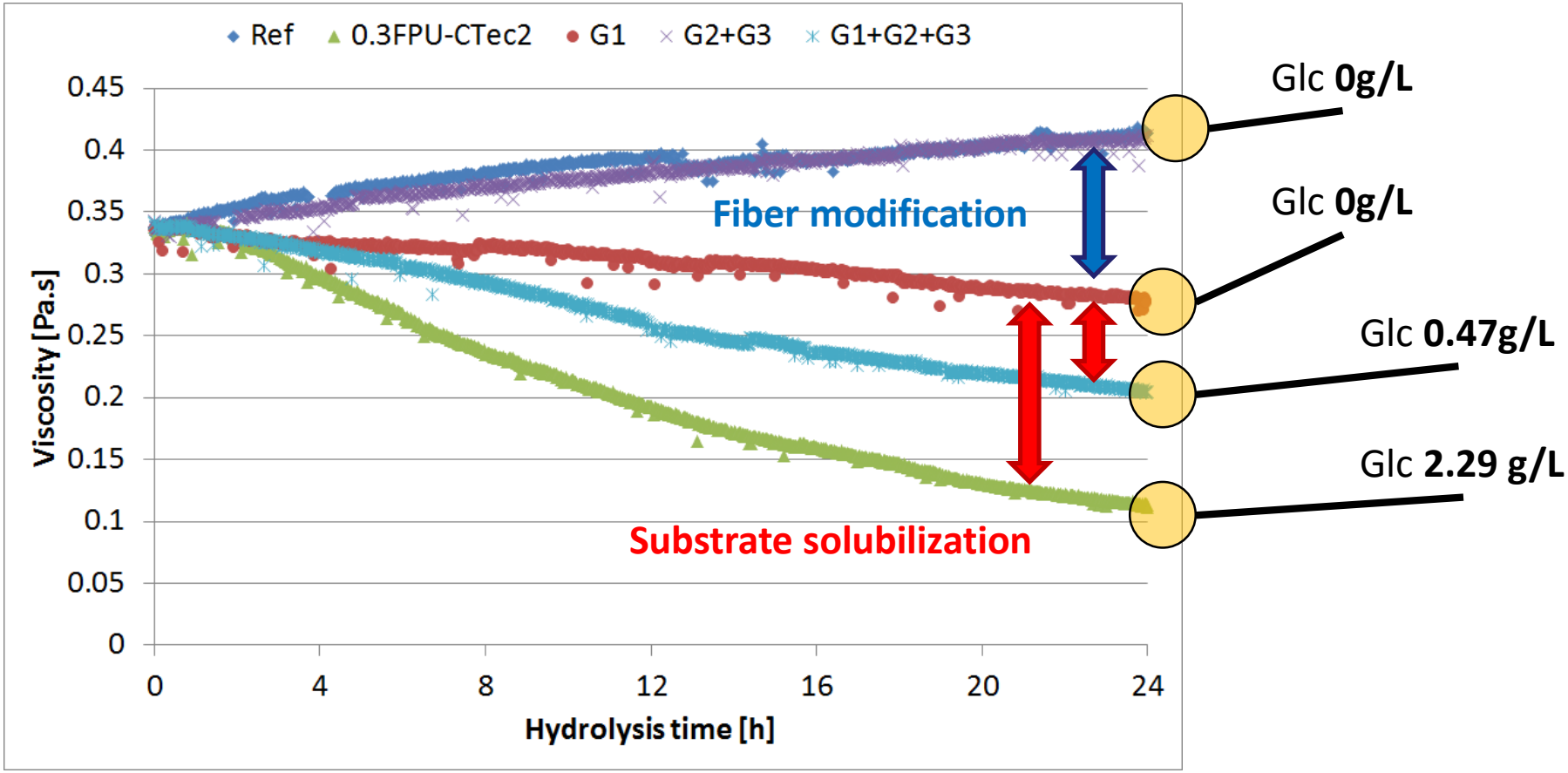
Glc **0g/L**

Glc **0.47g/L**

Glc **2.29 g/L**

Pure activities contribution for liquefaction

Paper pulp 30gdm/L



Conclusions

1. Endo-glucanase = main enzymes in the liquefaction of lignocellulosic suspension.

2. Exo-glucanase and β-glucosidase can improve liquefaction efficiency by synergist with Endo-glucanase.

3. The reduction of suspension viscosity is related to two phenomena:
   i. Fiber modification,
   ii. Solubilization of substrate.

4. The viscosity rising step on pretreated sugarcane bagasse is occurred with an evolution of population from fine to coarse. Its mechanism need to be investigated through alternatives experiments and analysis.

Thank for attention!