HIROSHIMA UNIVERSITY

Hydrothermal pretreatment of biomass for ethanol fermentation

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First and second generation ethanol

First generation

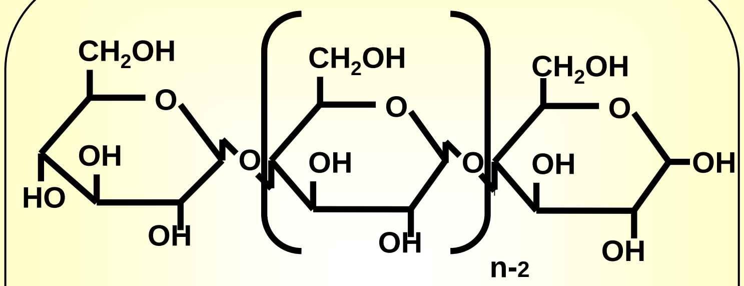
- Sugar, Starch, grain
- Easy fermentation to bioethanol
- High price raw material
- Competition with foods

Second generation

- Lignocellulosic residues (wood, straw) and other agricultural residues
- Advanced technology is needed

Saccharification of lignocellulosics

Lignocellulosics



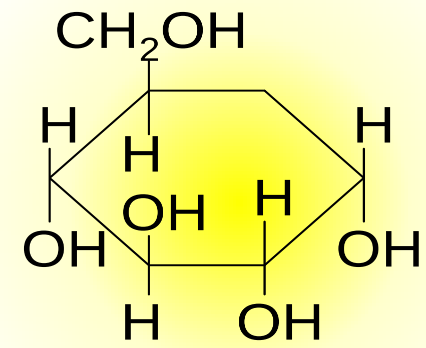
Cellulose

Hemicellulose

Lignin

Pretreatment

Enzymatic hydrolysis



Glucose

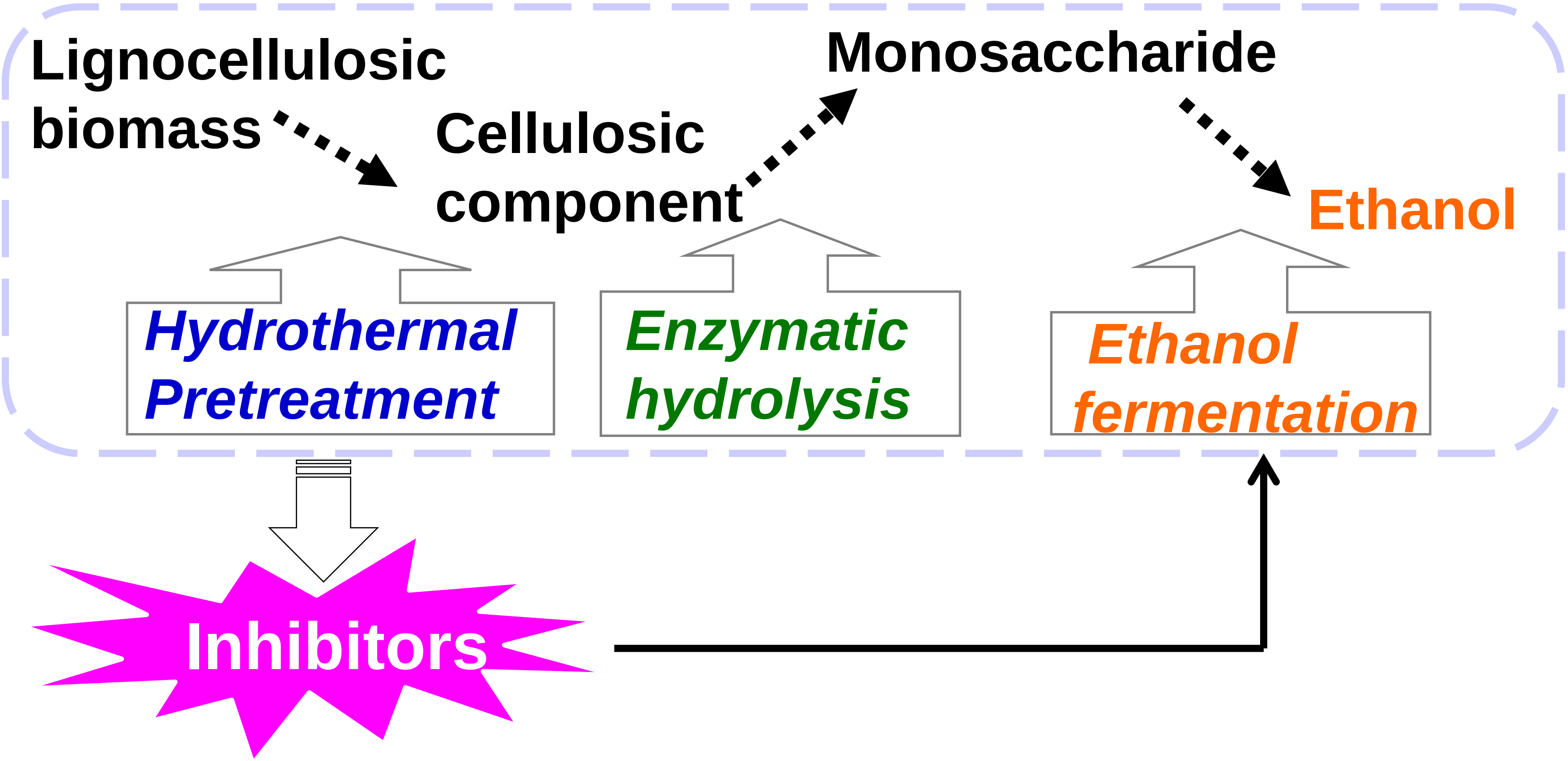
Ethanol fermentation

Ethanol

Various pretreatment for saccharification

Pretreatment	Concept	Disadvantage	Author (year) Previous study
Concentrated sulfuric acid	Promote hydrolysis with concentrated sulfuric acid	・Decomposition of glucose by acid ・ High cost to use acids	Gupta R et al. (2009)
Dilute sulfuric acid	Promote hydrolysis with dilute sulfuric acid	・High cost to treat byproducts ・Reactor corrosion	Root et al. (1959)
Steam explosion	After heating up in steam, suddenly reduce the pressure	・Low glucose yield	DeLong (1981)
Pulverization	Decrease the crystallinity of cellulose	・Large amount of energy needed.	Sidiras and Koukios (1989)
Hydrothermal	Hemicellulose is dissolved in water by high temperature and pressure. Reduction of crystallinity of cellulose.	・Low cost ・Low glucose yield	Mok and Antal (1994)

Inhibitor byproducts for fermentation

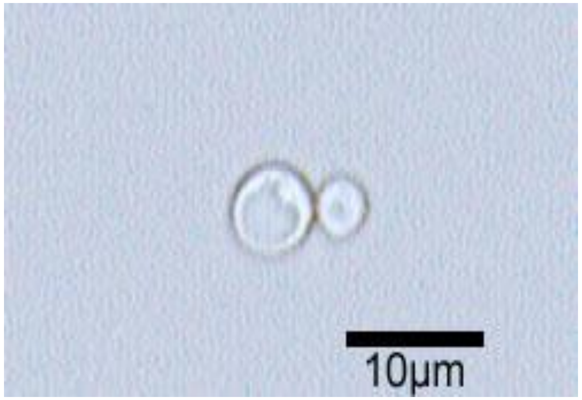


Fermentation inhibitors

<u>Yeast</u> ***Saccharomyces cerevisiae***

Aerobic ⇨ **Cell growth**

Anaerobic ⇨ **Ethanol fermentation**



Fermentation inhibitors are produced during hydrothermal pretreatment, which affects the activities of the yeast

Inhibitors ⇨ **Formic acid, Acetic acid, Furfural , 5-HMF**

Purpose

To commercialize the process, reaction characteristics as well as inhibitor effect should be clarified.

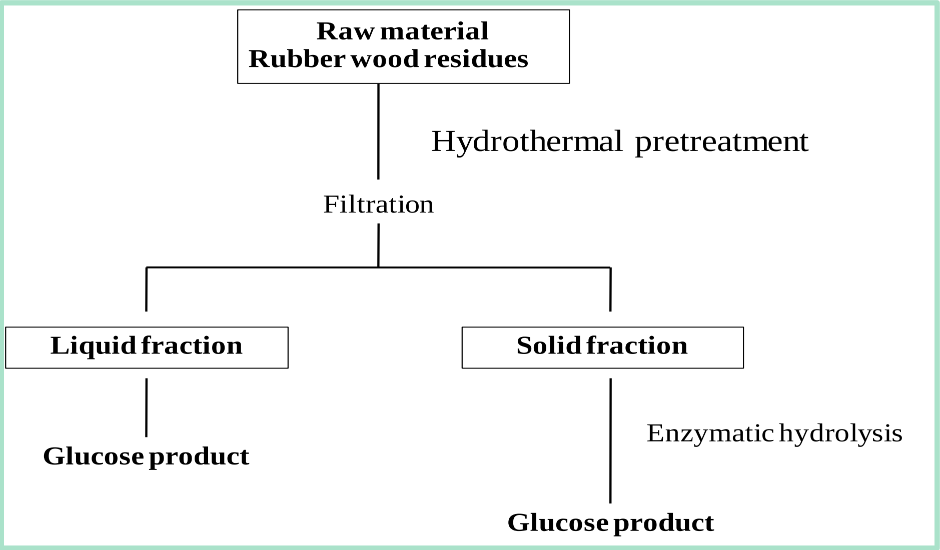
but・・・

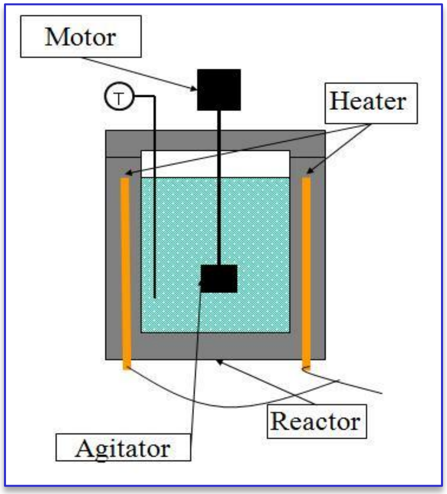
This evaluation has not been reported so far.

Purpose of this study

The purpose of this study is to determine the reaction characteristics and inhibitor effect quantitatively.

Experimental for hydrothermal pretreatment





Autoclave reactor

The working volume of the pretreatment vessel was 96 mL. The pretreatment agitator was set at 500 rpm.

Enzymatic hydrolysis

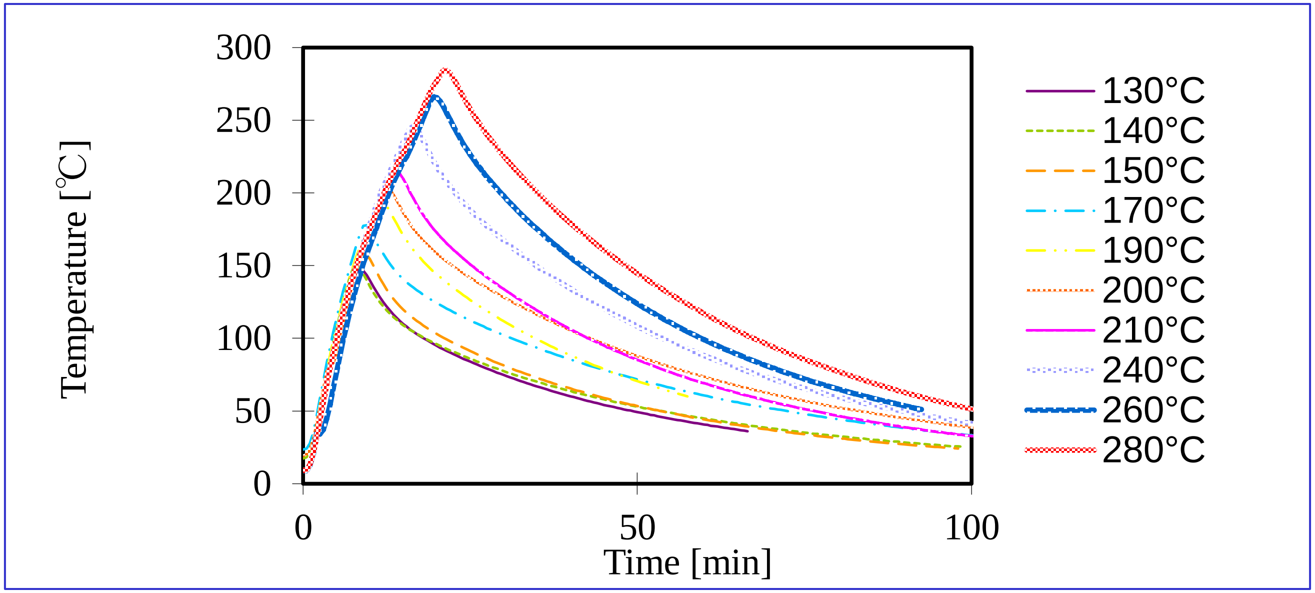
Reactant	1 g
Buffer fluid	60 mL
10 g/L cellulase solution	5 mL

Cellulase from *Aspergillus niger* powder, ≥0.3 units/mg solid

The flasks were shaken at 250 rpm at 37 C HPLC with SUGAR K S-802(Shodex) column operated at 60 ° C with 0.8mL/min flow of water as an eluate. The detector was a refractive index

Experimental conditions

Temperature	130, 150, 170, 190, 200, 210, 240, 260 and 280 °C
Rubber wood powder	7 g
De-ionized water	63 g



Temperature history for different target temperatures.

Feedstock

Rubber wood residue

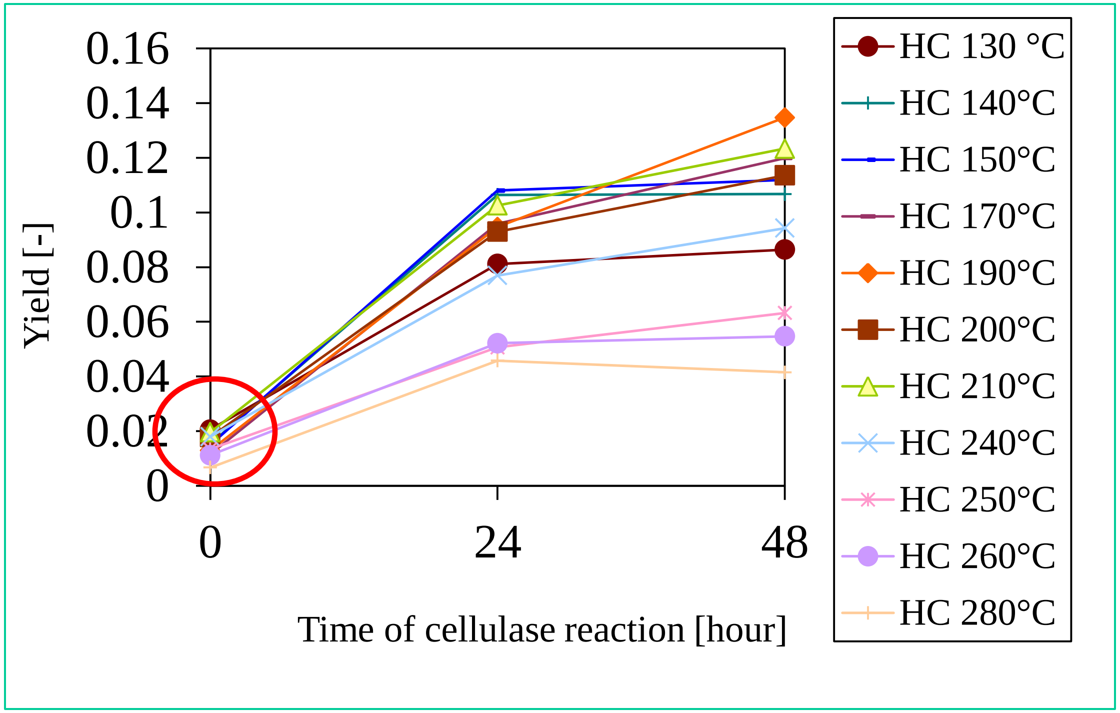
37% of market share export of the world are from Thailand

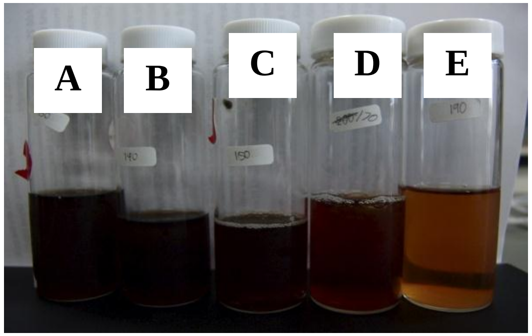
CHEMICAL CHARCTERISTICS OF RUBBER WOOD RESIDUE

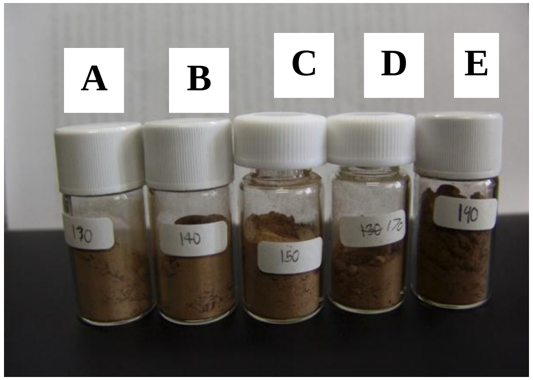
Composition	%
hemicelluloses	29
lignin	28
cellulose	39
ash	4

•United States Department of Agriculture, "Forage fiber analyses (Apparatus, reagents, procedures, and some applications)", *Agriculture Handbook*, 379 (1970)

Products and saccharification



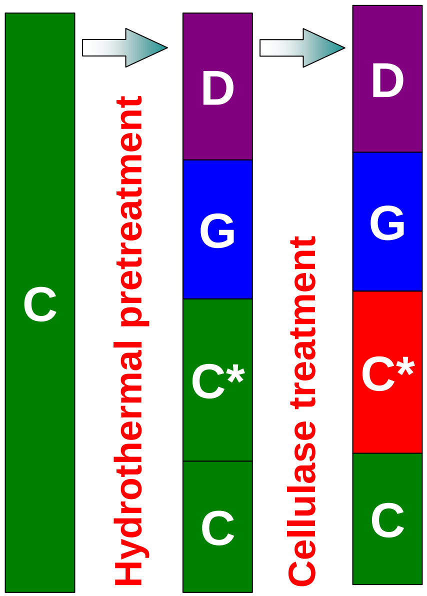


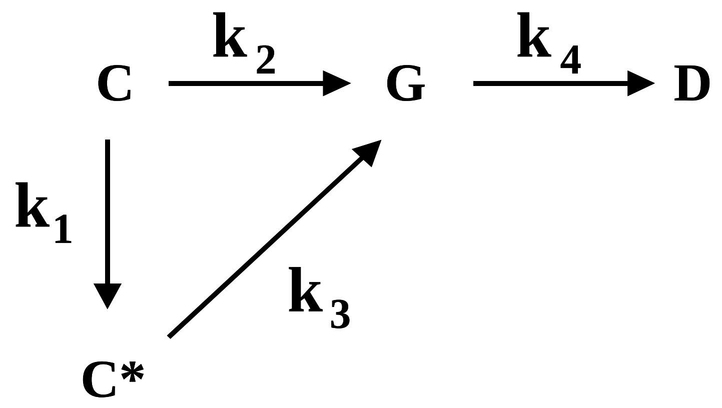


Time dependence of amount of glucose generated from solid residue treated temperature 130–280°C, 10 wt% of concentration of raw material, treatment time 0 min. (HC denote the solid sample from hydrothermal pretreatment used with enzymatic hydrolysis)

The samples after hydrothermal pretreatment at temperature on 130 (A), 140 (B), 150 (C), 170 (D), and 190° C (E)

Reaction modeling





$$k = A\,\exp(-\Delta E / RT)$$

C	Cellulose
C*	Cellulose hydrolyzed by cellulase after pretreatment
G	Glucose
D	Decomposition products of glucose

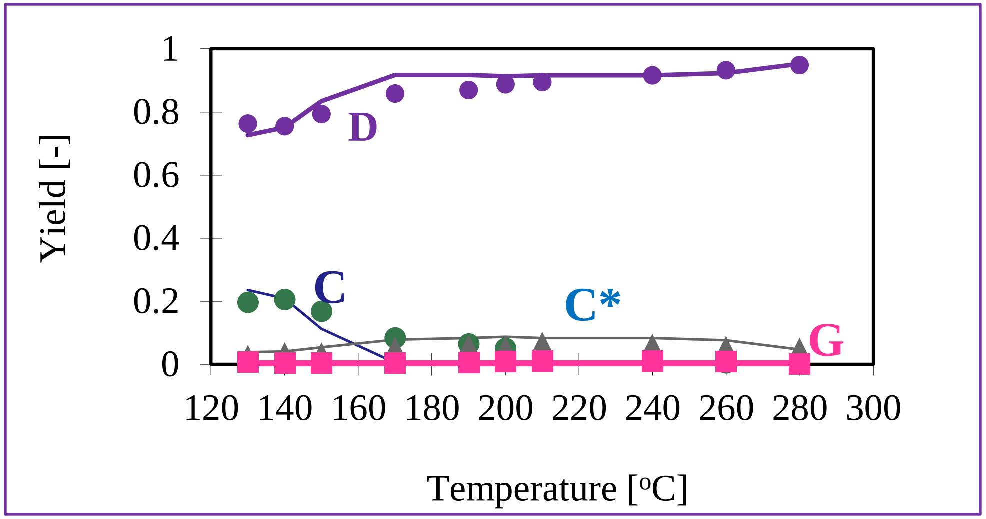
Reaction rate parameters

$$\frac{d[C]}{dt} = -k_1[C] - k_2[C]$$

$$\frac{d[C^*]}{dt} = k_1[C] - k_3[C^*]$$

$$\frac{d[G]}{dt} = k_3[C^*] + k_2[C] - k_4[G]$$

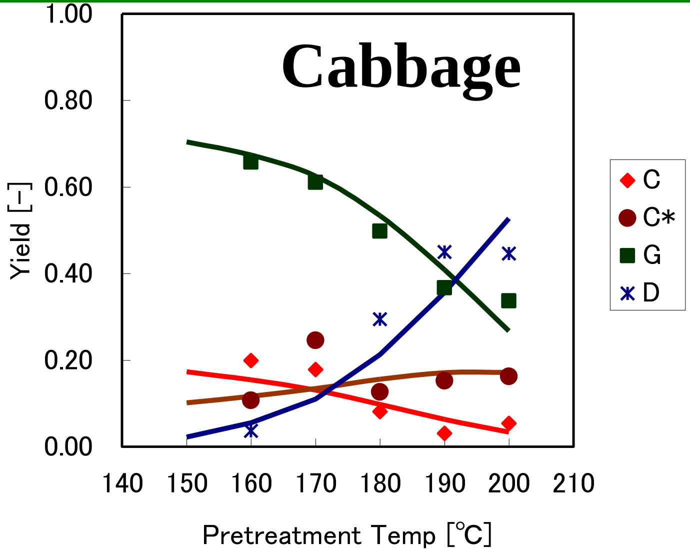
$$\frac{d[D]}{dt} = k_4[G]$$

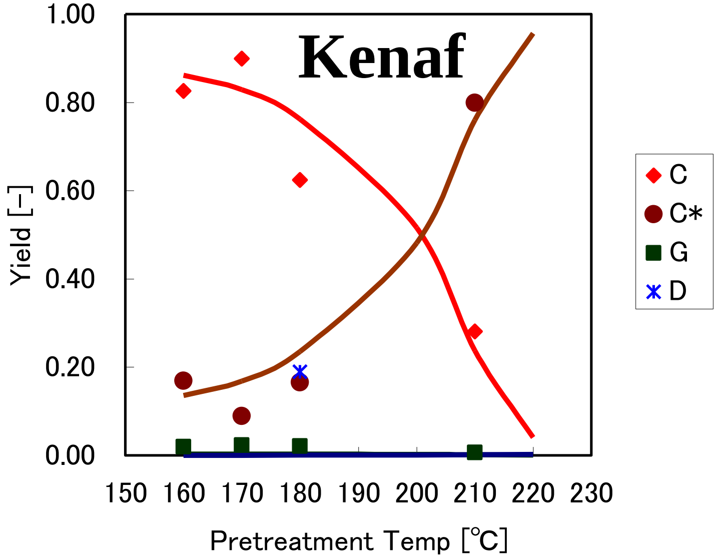


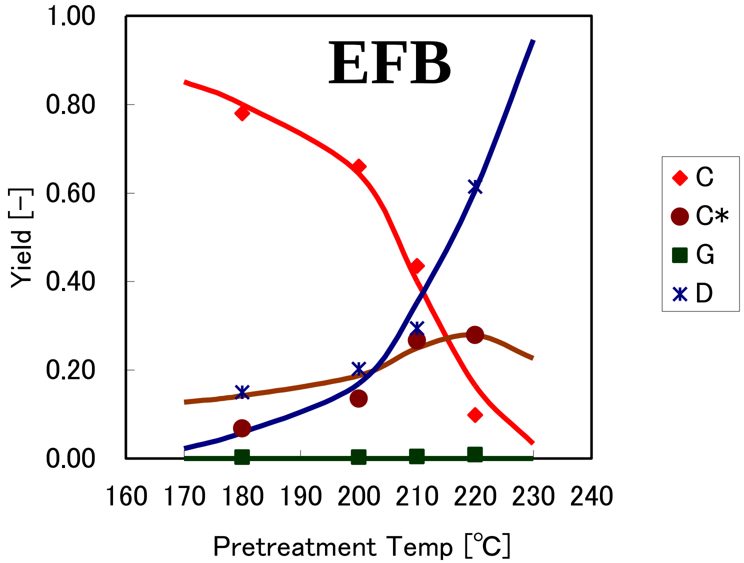
Reaction rate parameters

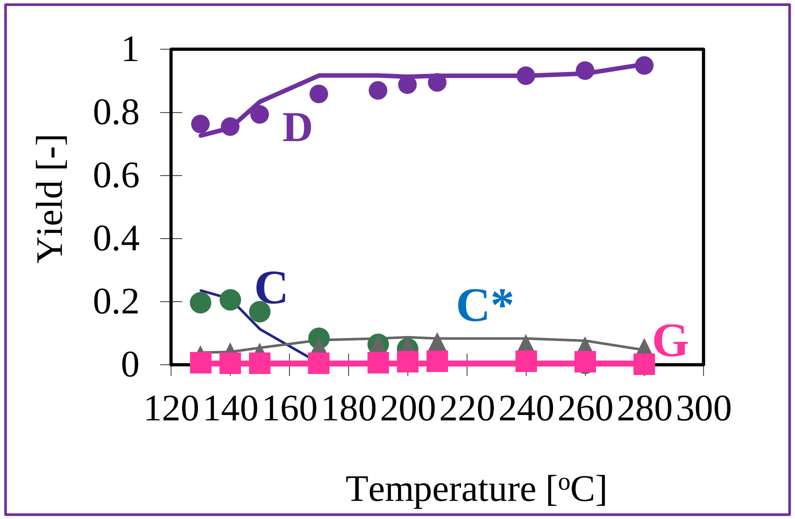
	Preexponential factor [1/s]	Activation energy [kJ/mol]
k_1	1.87×10^5	62.0
k_2	2.02×10^7	87.7
k_3	1.80×10^{18}	222.2
k_4	2.88×10^2	14.4

Comparison with other feedstocks









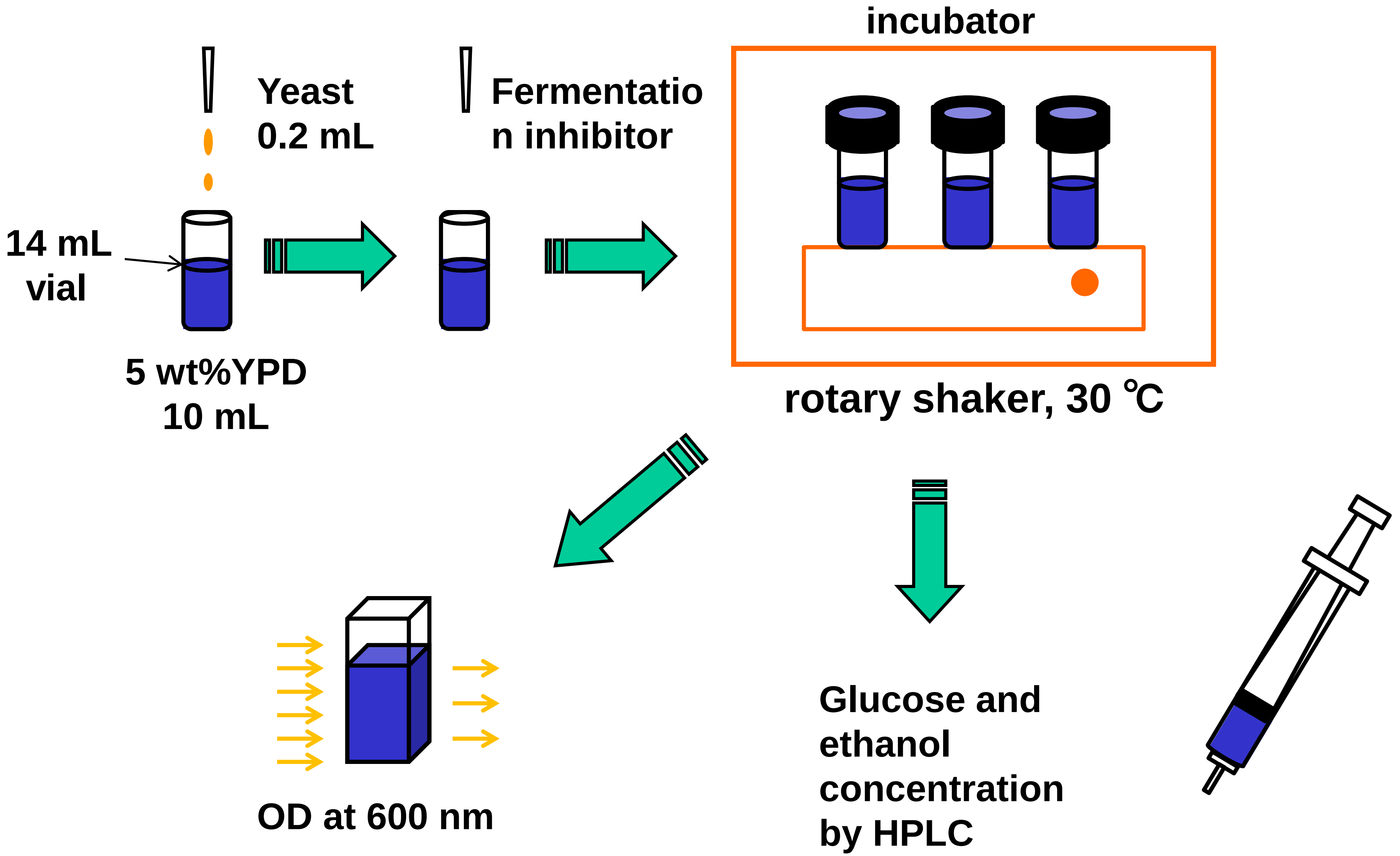
Conclusions (reaction characteristics)

Model for the reactions in hydrothermal pretreatment reactor was proposed.

The reaction parameter in the hydrothermal reactor for rubber wood was successfully decided.

Reaction characteristics differs from feedstock to feedstock.

Experiment for inhibitor effect clarification



Experimental conditions

Yeast	*S. cerevisiae* *
YPD medium (5.0 wt%)	10 mL
Preculture	0.2 mL
Inhibitor concentration	
Formic acid	0-45 mM
Acetic acid,	0-45 mM
Furfural	0-45 mM
5-HMF	0-15 mM
Measuring time	36 h
Incubation temperature	30 ^{o}C

*Sigma-Aldrich (Type II)

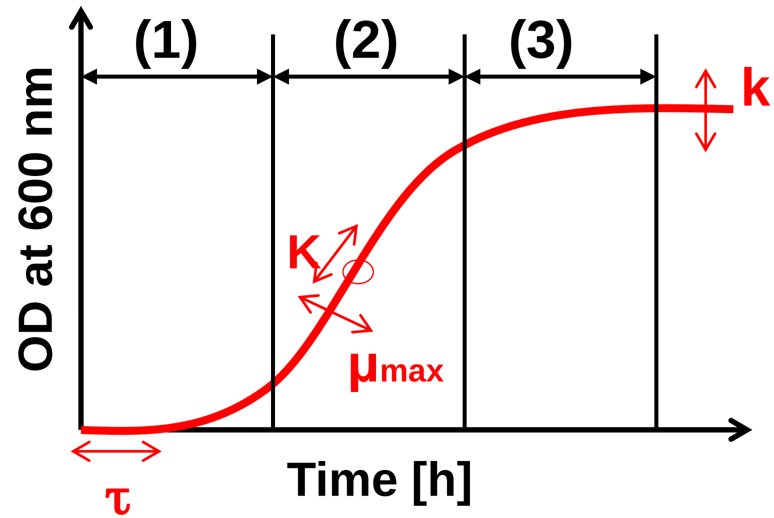
Cell growth model

$$\mu=\mu_{max}\frac{S}{S+K}$$

(Monod equation)

$$\frac{dX}{dt}=\mu X$$

$$\frac{dS}{dt}=-k\frac{dX}{dt}$$



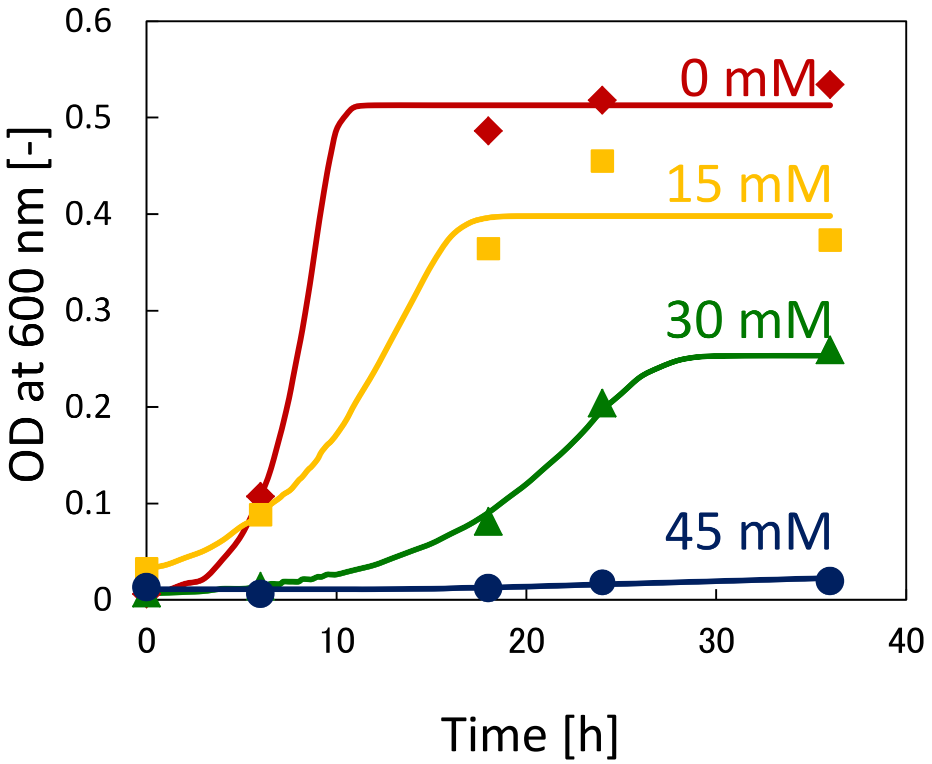
(1) Lag phase (2) Exponential growth phase
(3) Resting phase

$$(t-\tau)=\frac{1}{\mu_{max}}\left\{\left(1+\frac{K}{kXo+So}\right)\ln\left(\frac{X}{Xo}\right)-\frac{K}{kXo+So}\ln\left|1+\frac{k}{So}(Xo-X)\right|\right\}$$

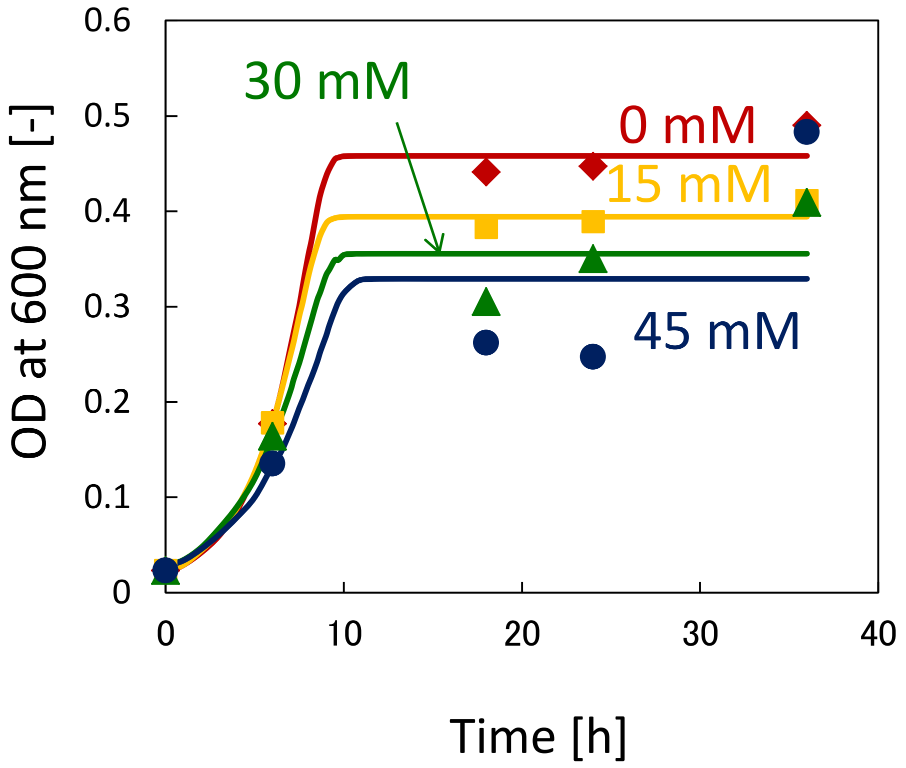
X : Cell concentration
X_0 : Initial Cell concentration
S : Culture medium concentration
S_0 : Initial culture medium concentration
t : Incubation time

μ_{max}: Maximum growth rate
K : Half medium concentration rate
k :
τ: Lag phase time

Inhibitor effect on cell growth

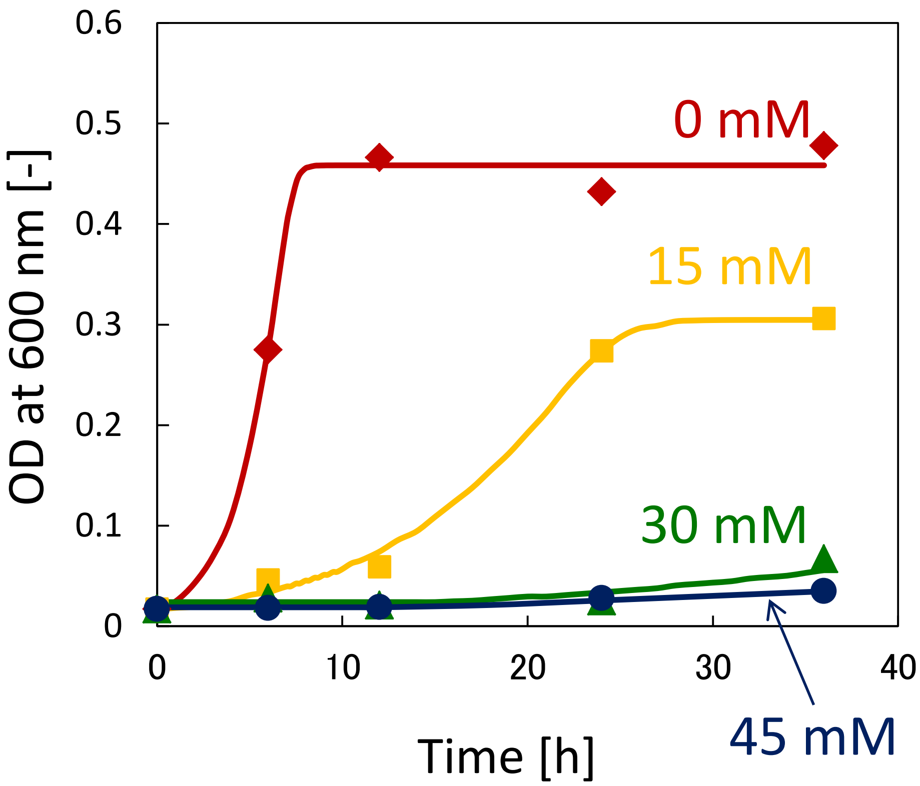


Formic acid

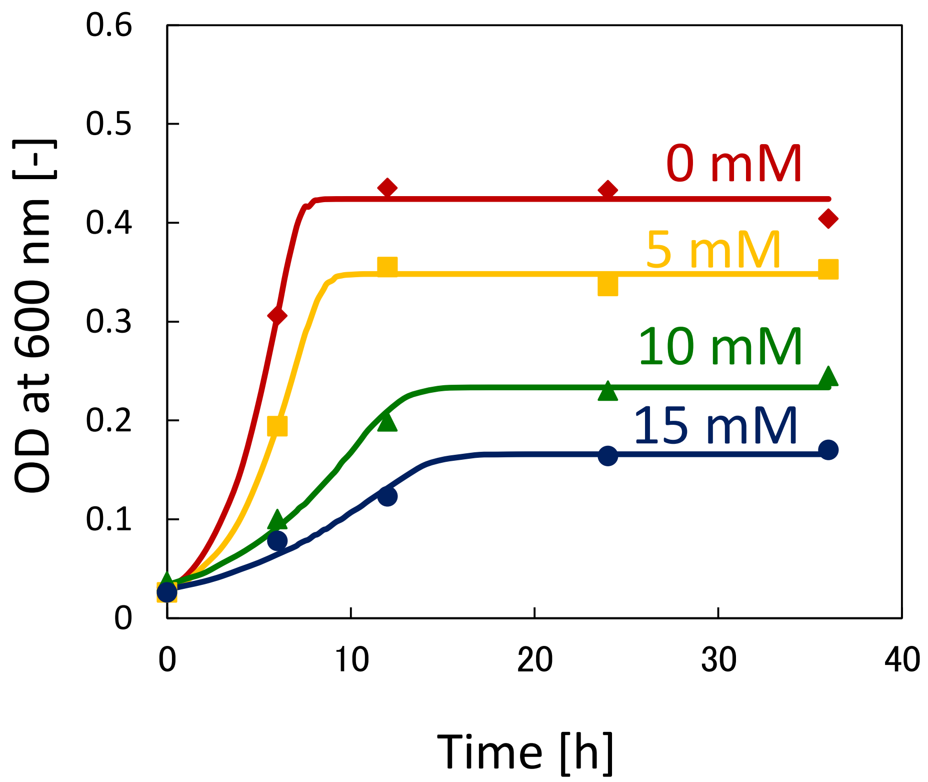


Acetic acid

Inhibitor effect on cell growth

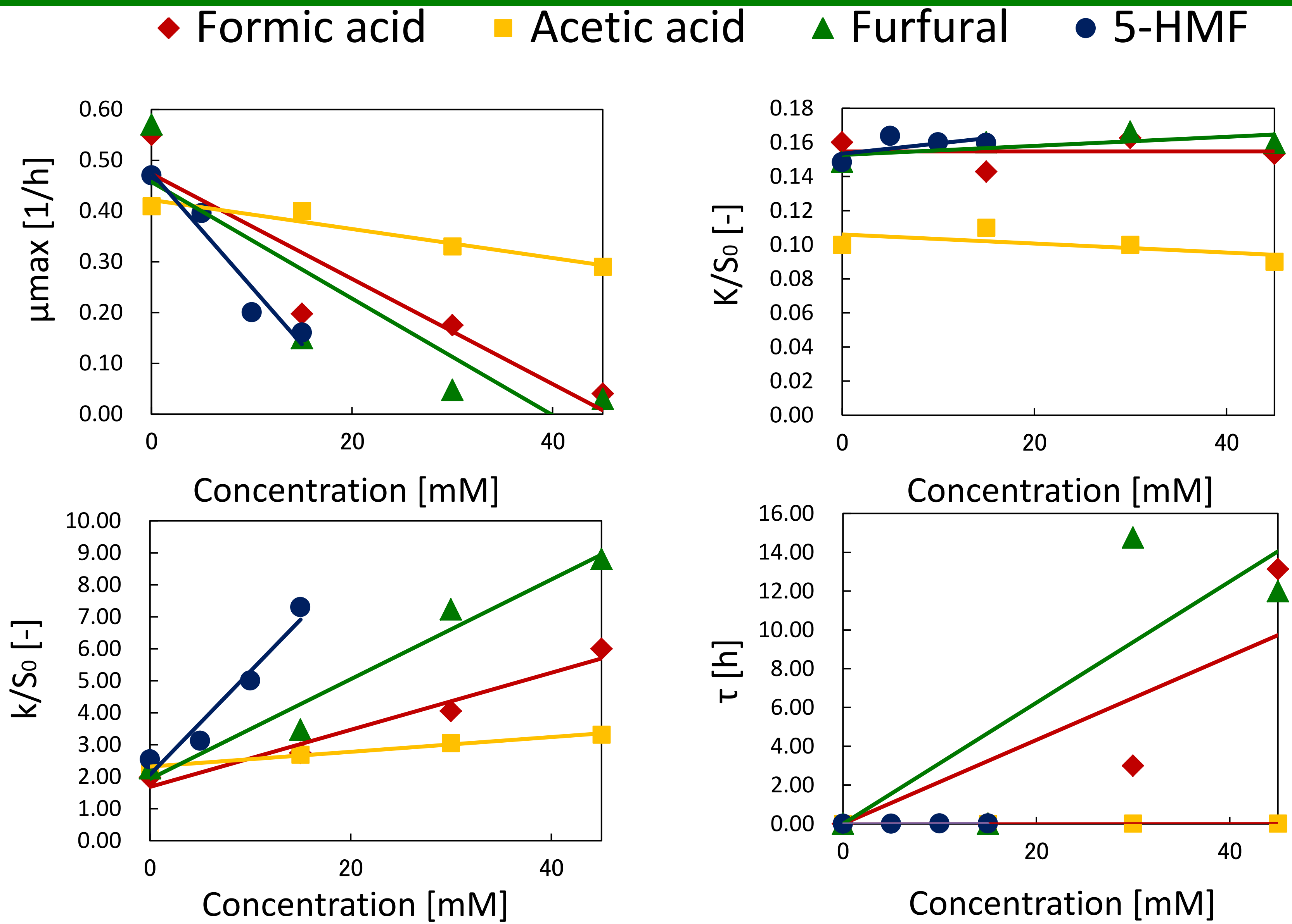


Furfural

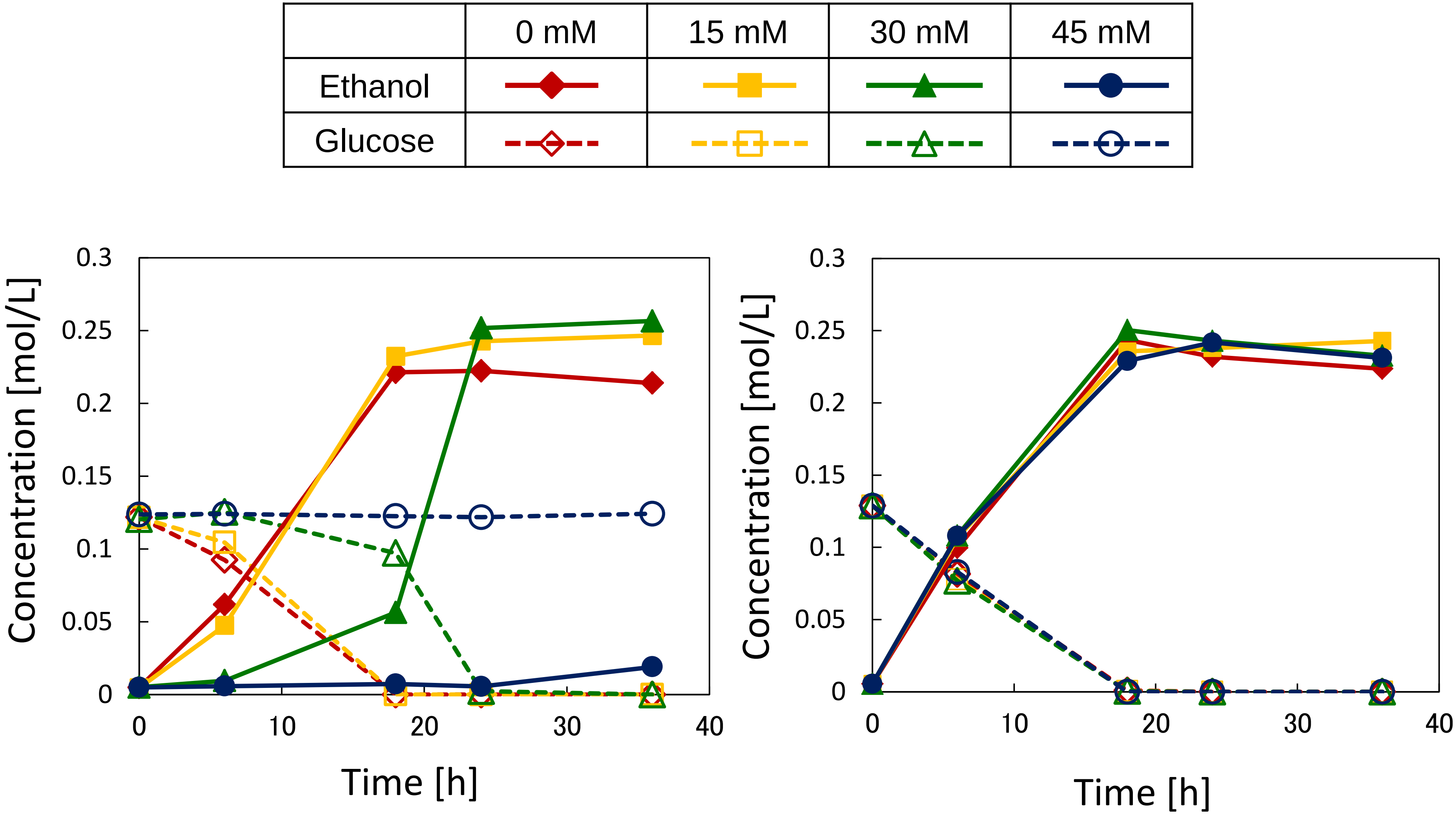


5-HMF

Monod parameter change by inhigibors



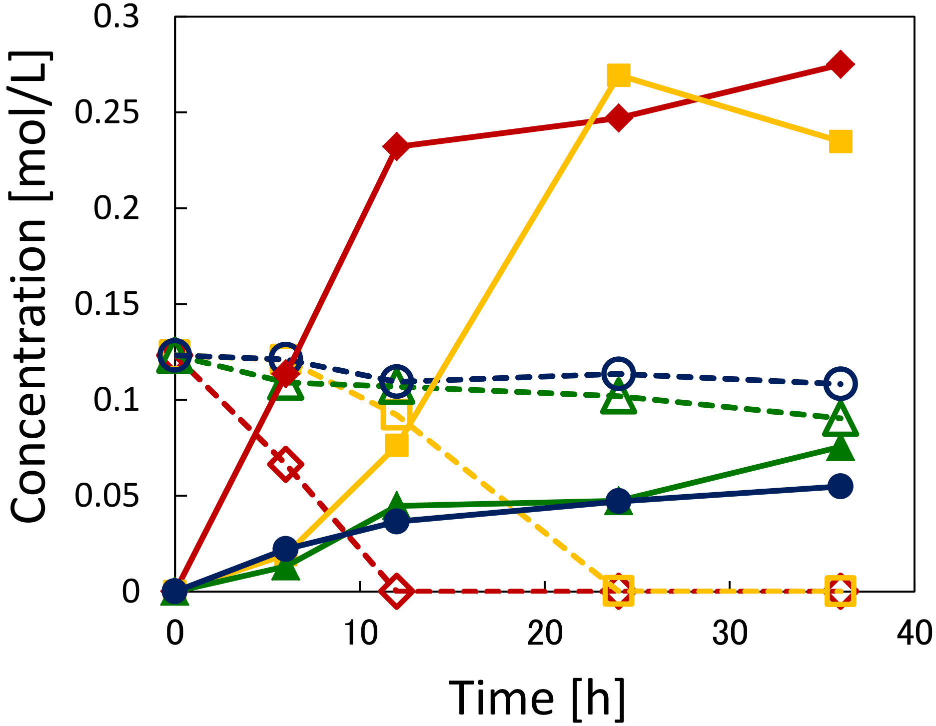
Inhibitor effect on ethanol fermentation



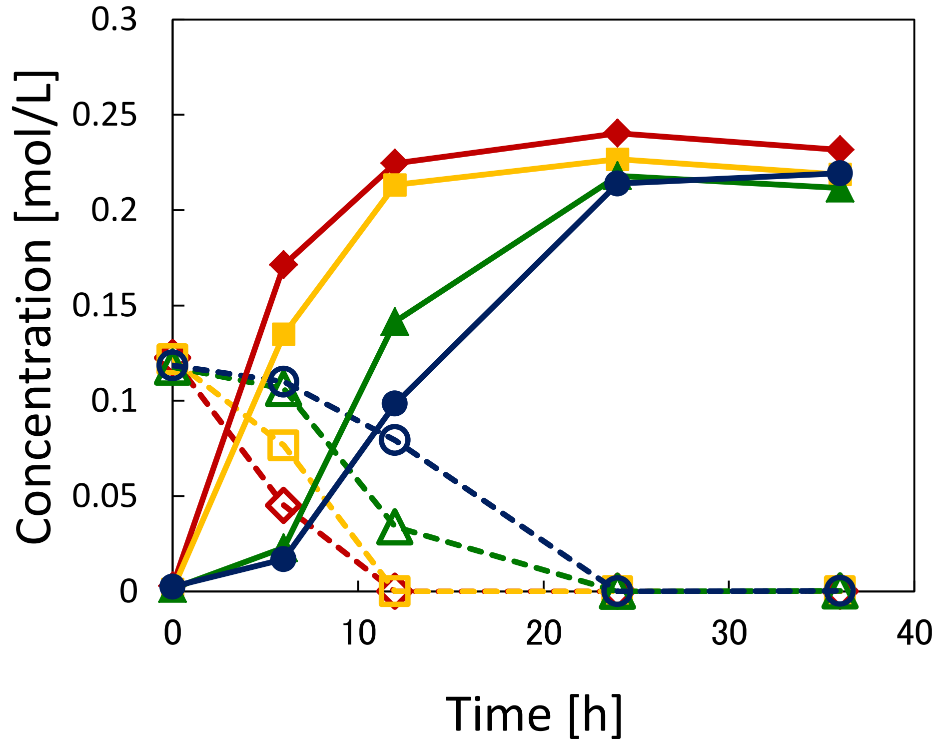
Formic acid **Acetic acid**

Inhibitor effect on ethanol fermentation

Furfural	0 mM	15 mM	30 mM	45 mM
5-HMF	0 mM	5 mM	10 mM	15 mM
Ethanol	—◆—	—■—	—▲—	—●—
Glucose	--◇--	--□--	--△--	--○--



Furfural



5-HMF

Conclusions (inhibitor effect)

- The inhibitors used in this study slows cell growth and final yeast concentration. Effect on parameters were observed (μ_{max} , k, τ).

- The inhibitors used in this study except acetic acid decreases glucose consumption rate and ethanol production rate for ethanol fermentation.

- Acetic acid affects cell growth but does not affect ethanol production.

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Thank you!!

See you at the European Biomass Conf. and Exhibition, 3-7 June 2013
Bella Center - Copenhagen, Denmark