IOWA STATE UNIVERSITY

Food Science and Human Nutrition Department

# Bioprocessing of Agri-food Coproducts for higher-value Biochemicals and Bioproducts: Some Research in Lamsal Lab


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# Presentation Outline:

- Quick self-introduction and my research areas
- Bioprocessing
- Research example
- Discussion

# A Little Bit About Myself:

- From Syangja bazaar, Nepal
- BS: Ag. Engineering, 1992, Tamil Nadu Ag. University, India
- MS: Post-Harvest Technology, 1994, Asian Institute of Tech. Bangkok, Thailand
- PhD: Ag. Engineering, 2004, UW-Madison; research in ARS/USDFRC Madison Lab
- Professor, *Food Processing, FSHN department, Iowa State University: 2008- present*
- Professional Engineer (PE)- Agricultural Engineering: *2009- present: State of Iowa*

# My Long-Term Research Goals

The basic premise of my research is to apply physical, chemical and biological principles for complete and novel uses of agricultural resources, especially, crops, their components, and co-products to add value, and create novel and sustainable processes and products

- *Extraction, fractionation*
- *Enzyme-aided processing*
- *Fermentation*
- *Extrusion*

# Iowa State University: 2008- present : Faculty

## Two Broad Areas of Research:

1. **Bioprocessing for biobased products/ chemicals**
   - Green Chemistry; Sustainable Value-added utilization of agricultural resources for food, feed and biobased materials/ chemicals
     - Tools: fermentation, enzymes applications, extrusion etc.
     - Examples:
       - Protein hydrolysates; Probiotic cereal brans; Antifreeze Protein (AFP); Microbial Biosurfactants

2. **Plant Protein ingredients for food, feed, and industrial applications and processing impact**
     - Tools: Extrusion, ultrasonication, Cold-plasma, enzymes etc.
     - Examples:
       - Corn and Soy proteins; Dry bean proteins, Milk protein; Insect Proteins

# Adding Value to Agri-food Resources through Bioprocessing

- **What comes to your mind seeing this picture?**
- **What Engineering disciplines may have been involved?**

# Biomanufacturing/ Bioprocess Engineering

- Bioprocessing/ biomanufacturing is the process of using living organisms or their components to create products that can be food, pharmaceuticals, or chemicals.

- It utilizes living organisms, such as cells, viruses, or microorganisms that are grown in suitable media in vessels called bioreactors under conditions that are ideal for producing the desired product including biochemicals.
  - Examples in food- cheese, yogurt, bread, wine, and beer
  - In pharmaceuticals: vaccines, painkillers, antibiotics and drugs
  - In chemicals- biofuels and chemicals from renewable resources

# Bioprocess Engineering:

**Bioprocess engineers** use their knowledge of biology, chemistry, and mathematics to design, develop, and improve biological systems and processes (which is engineering).

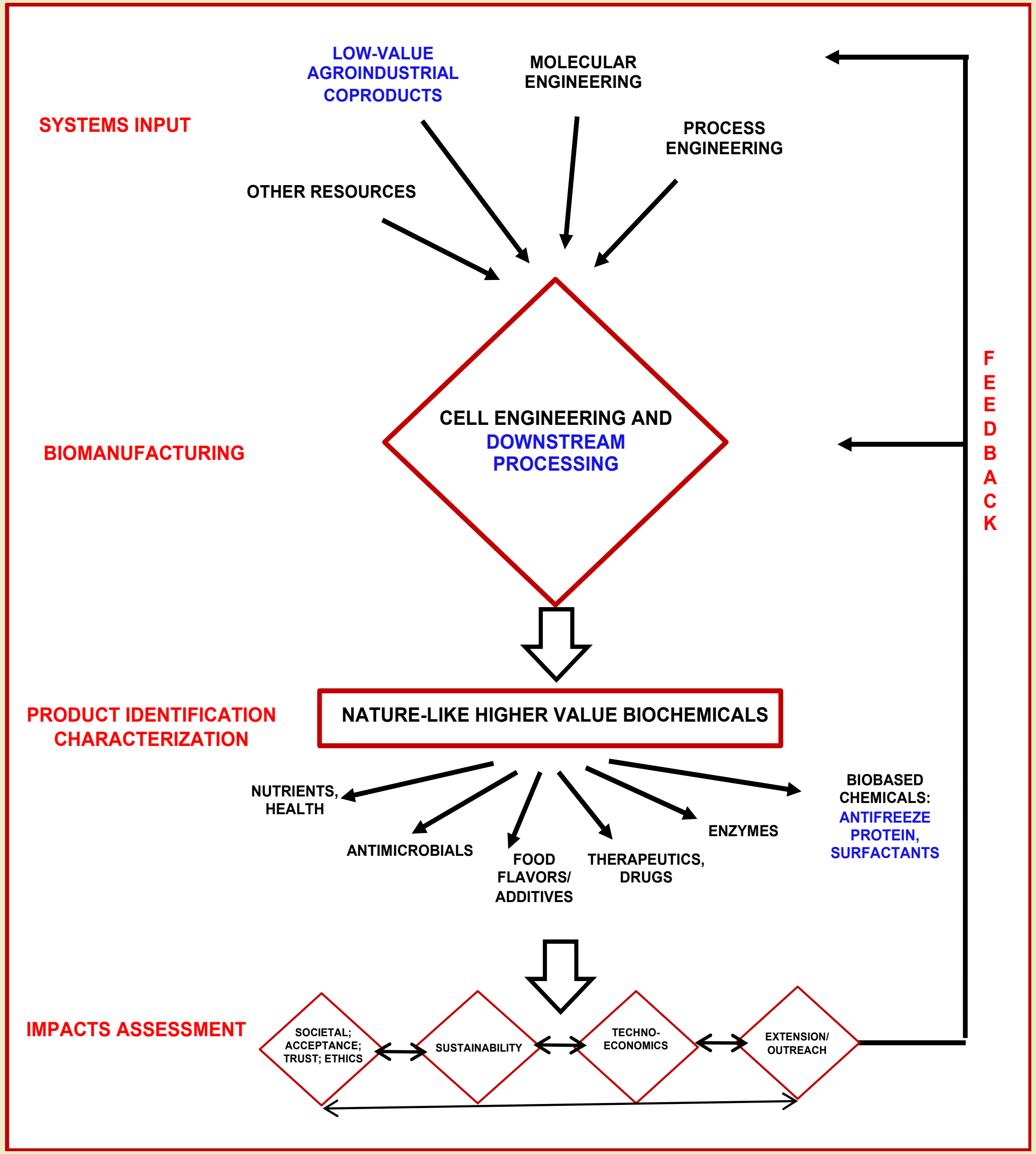
- **Design and develop**: Create processes for producing biological materials, such as medicines, food, or fuel
- **Optimize**: Improve efficiency and reduce costs for existing processes
- **Conduct research**: Test new production methods and analyze data to monitor performance
- **Ensure compliance**: Make sure processes meet regulatory standards and safety guidelines
- **Scale up**: Move processes from the lab to full-scale production
- **Maintain equipment**: Troubleshoot and maintain equipment used in bioprocessing
- **Develop safety protocols**: Ensure a safe working environment

## Bioprocessing Industries (Clientele)

- **Pharmaceuticals:** Design medicines that improve health outcomes and limit side effects
- **Food:** Design ways to process food on a large scale, and improve its nutritional value
- **Fuel:** Design and optimize processes for producing biofuel
- **Waste management**: Design processes to manage waste
- **Biotechnology**: Work in biotechnology firms to develop new bioproducts and biochemicals
- **Agriculture:** Design hybrid plants and chemical treatments to protect crops

# Bioprocessing: a Multidisciplinary enterprise

- Cell/ Bioengineering: upstream gene manipulation
- Cultivation/ Fermentation
- Downstream Separation/ isolation of compounds of interest
- Biochemistry (of target molecule- protein, other biochemical)- for functional applications
- Impact assessments: TEA, societal, sustainability
- Industry collaborators (later stages- ultimate users)



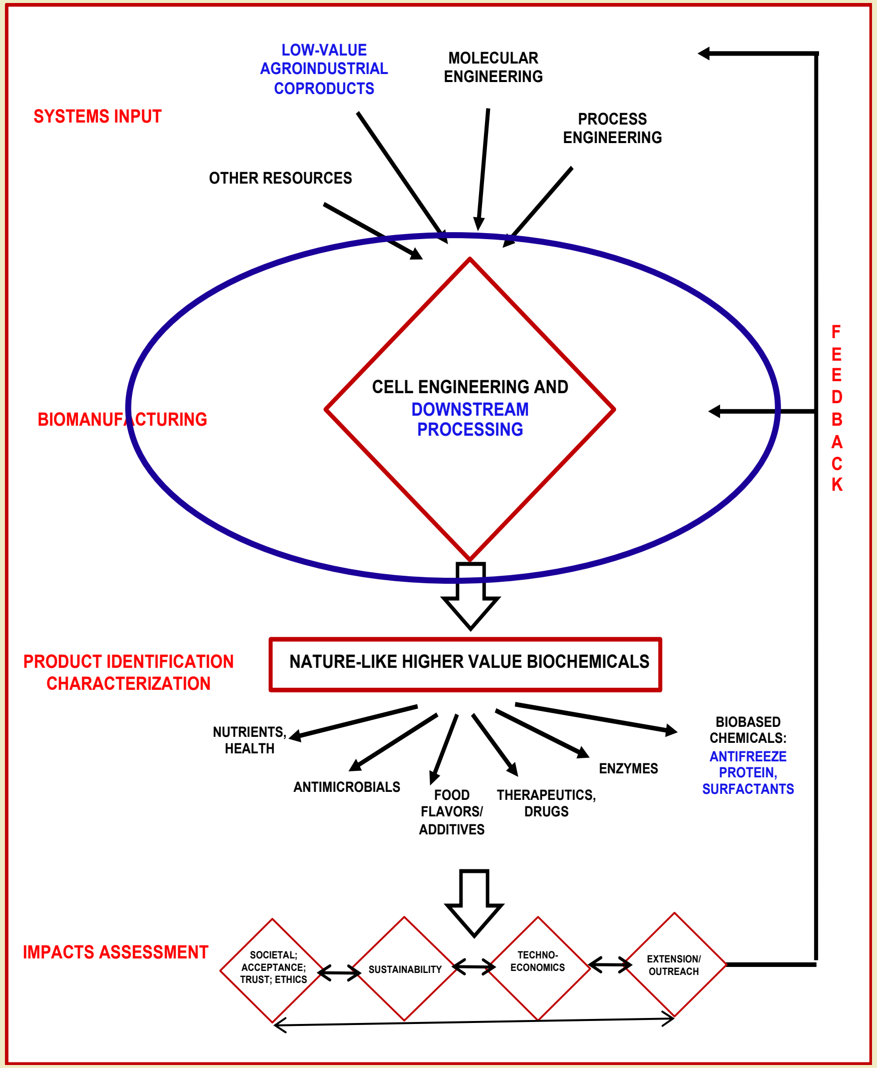
# Bioprocess Engineering AKA Biochemical engineering

- The field of study has roots stemming from chemical engineering and biological engineering
- Deals with the design, construction, and advancement of unit processes that involve biological organisms (such as fermentation) or organic molecules (often enzymes)
- Has various applications such as biofuels, food, pharmaceuticals, biotechnology, and water treatment processes.
- The role of a biochemical engineer is to take findings developed by biologists and chemists in a laboratory and translate that to a large-scale manufacturing process


Bioreactor

# Bioprocess Engineering: contributing disciplines

- Chemical Engineering
- Biological Engineering
- Process Engineering and control
- Food/ Ag. Engineering
- Mechanical Engineering (equipment design, fluid flow)
- Structural Engineering



# Degrees in Bioprocess Engineering:

- University of Illinois Urbana-Champaign: BS in Bioprocess Engineering and Industrial Biotechnology
- State University of New York, Syracuse, NY: Dept. Chemical Engineering: BS in Bioprocess Engineering
- Florida A&M University (FAMU): BS in Biological Systems Engineering (BSE) with a Bioprocessing and Food Engineering option.
- The University of Queensland: BS in Bioprocess Engineering
- University of Canterbury, New Zealand: BS in Bioprocess Engineering
- Iowa State University: Biomedical Engineering; Chemical Engineering
- University of California- Berkley; Master of Bioprocess Engineering (MBPE) degree is 9-month degree program
- Tufts School of Engineering: MS in Bioengineering (Cell and Bioprocess Engineering Option)

# Research Example: Fermentation of corn steep liquor for functional protein (anti-freeze protein)

*Bibek Byanju, Swastik Sen, Thomas Mansell and **Buddhi P. Lamsal***: J Sci Food Agric 2023; 103, Journal of The Science of Food and Agriculture*

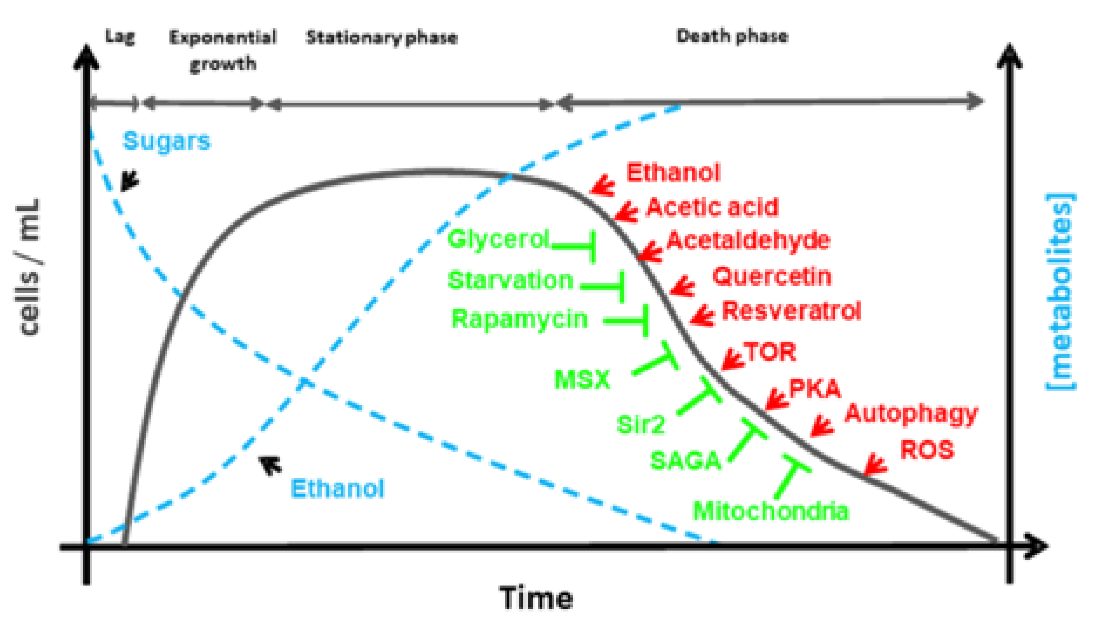
# What is Fermentation?

- The process in which a substance breaks down or converted into a simpler substance. Microorganisms like yeast and bacteria usually play a role in the fermentation process, creating beer, wine, bread, kimchi, yogurt and other foods.

- Both an aerobic or anaerobic process in which energy can be released from glucose into making of another biomolecule

- Fermentation parameters are factors that affect the outcome of fermentation, such as temperature, pH, and aeration, enzyme or microbes loading, media formulation etc..
  - Impact the growth of cells, the production of target compounds, and the solubility of proteins and amino acids.

- **Process Engineers need to control this reaction process**




Growth phases of wine yeast during grape juice fermentation, changes in key metabolites and main aging regulators. Curves of cell viability (grey line), sugar consumption and ethanol production (blue dotted lines) during the fermentation times are shown. Positive regulators of the chronological life span are indicated in green and pro-aging factors are indicated in red.

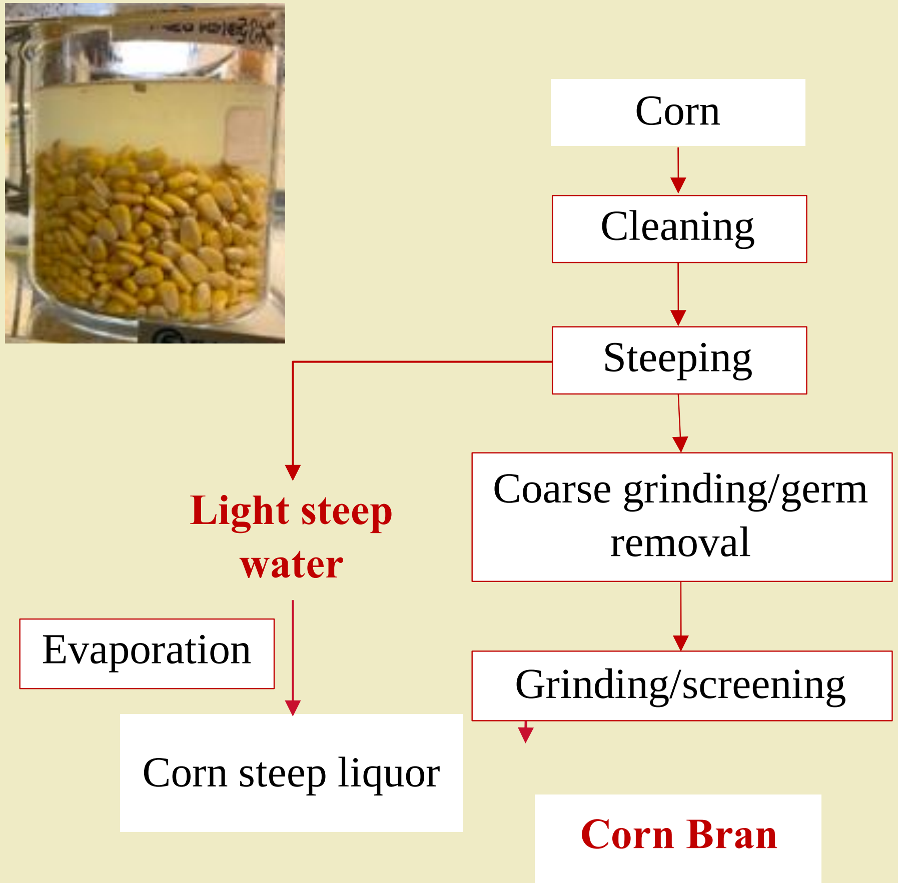
# Corn steep liquor

Light steep water (1: 4 water)
(~6-10% solids) :

- Soluble protein,  carbohydrates, minerals

- Evaporated to ~35% solids and Added to corn fibers➔ feed

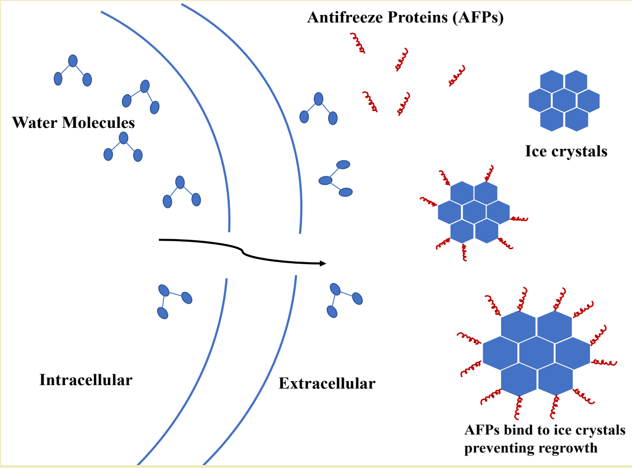
- **Proposed to utilize as microbial feed**



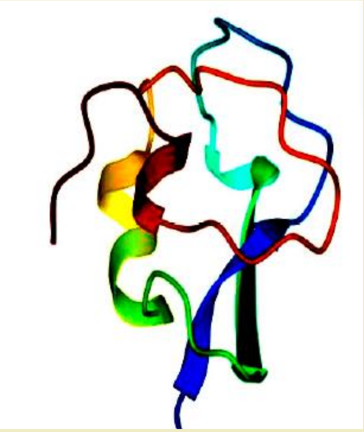

*Adapted from Rausch et al., 2006*

# Anti-freeze proteins (AFP)

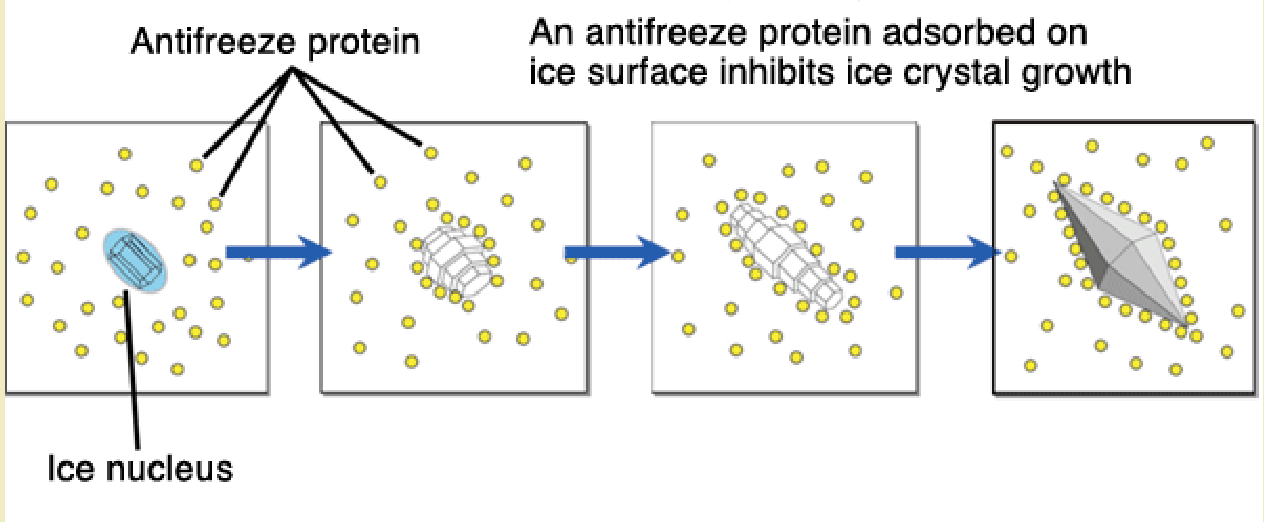
- Modify growth of ice crystals and depress the freezing point of water, resulting in stabilization of ice crystals as well as inhibition of ice re-crystallization.
- Found in polar fish, plants, insects, fungi, and bacteria.
- Market value $10 / mg (A/F Protein Canada Inc.)




*Schematic of AFP, shown as coils, binding to ice crystal lattices, preventing regrowth*



AFP III: Ocean pout: (6-7 kDa)




*Phillips and Williams, 2011*

- **AFP applications:**
  - Ice-cream, frozen meat, frozen dough.
  - Cryopreservation of cells, tissues, and organs.
  - Deicing of roads, aeroplanes

- **Challenges from natural resources**
  - Difficulties in extraction from natural resources
  - Not cost effective.
  - 1–4 g/ L of fish blood.

- recombinant technology and fermentation of agri-food coproducts to produce AFPs

*Ustun et al., 2015, Voets, 2017*

# Cell Bioengineering of *Lactococcus lactis* to produce AFPIII

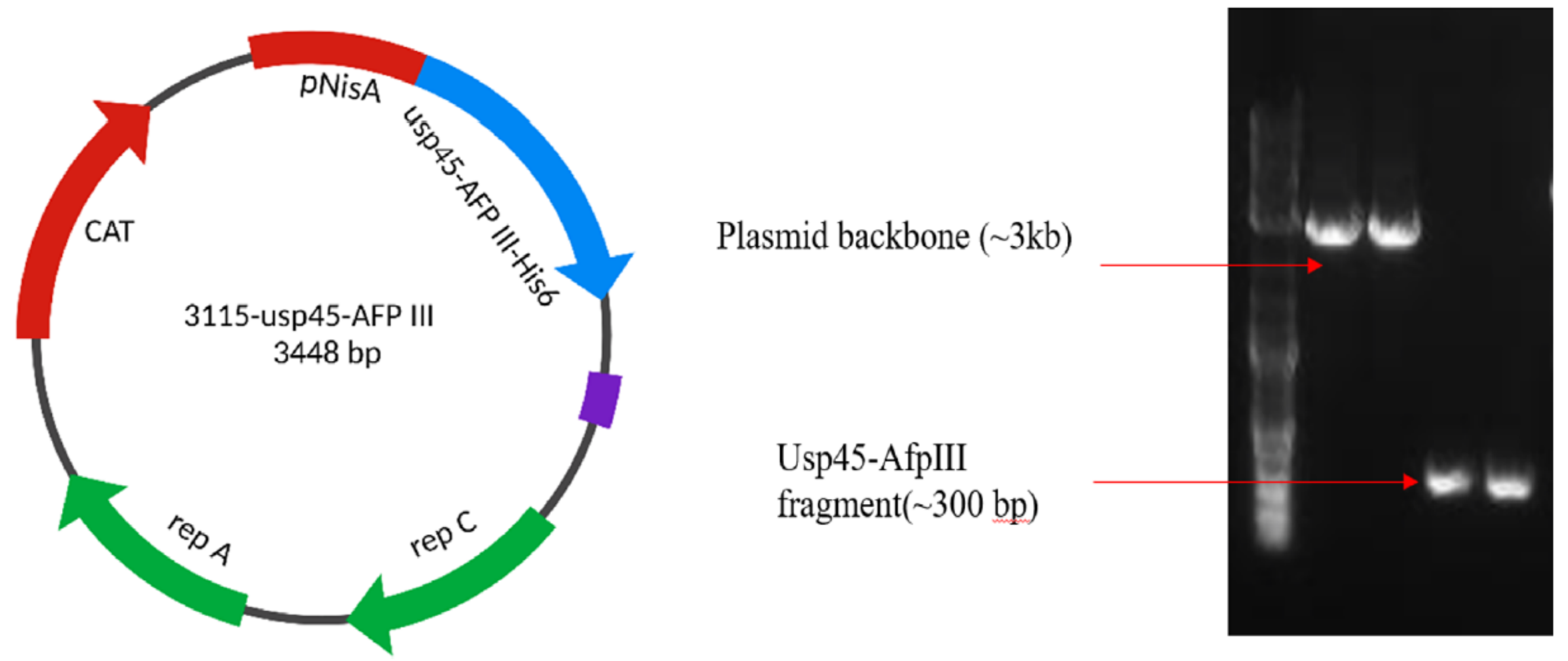



**Figure 1.** The plasmid construct (left) and amplified PCR products of the vector backbone and the USP 45-AFPIII fragments (right) of Ocean Pout AFP III.

# Media optimization for optimal fermentation of recombinant cells

Corn coproducts
(CSL and thin stillage)

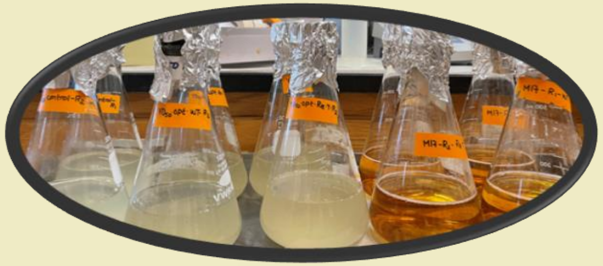
Optimization of media using Bio-screen (wild and recombinant *L. lactis*)

**Best optimized media**

Shake flask fermentation (250-mL)
(pH 6.9 ± 0.2, 30°C, Agitation-200 rpm for 72 h)

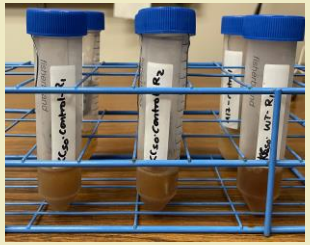
Additives/ trace elements

- Ascorbic acid (AA)
- Magnesium sulfate (MS)
- Disodium glycerophosphate
- Tryptone
- Zinc (Zn)
- Iron (Fe)



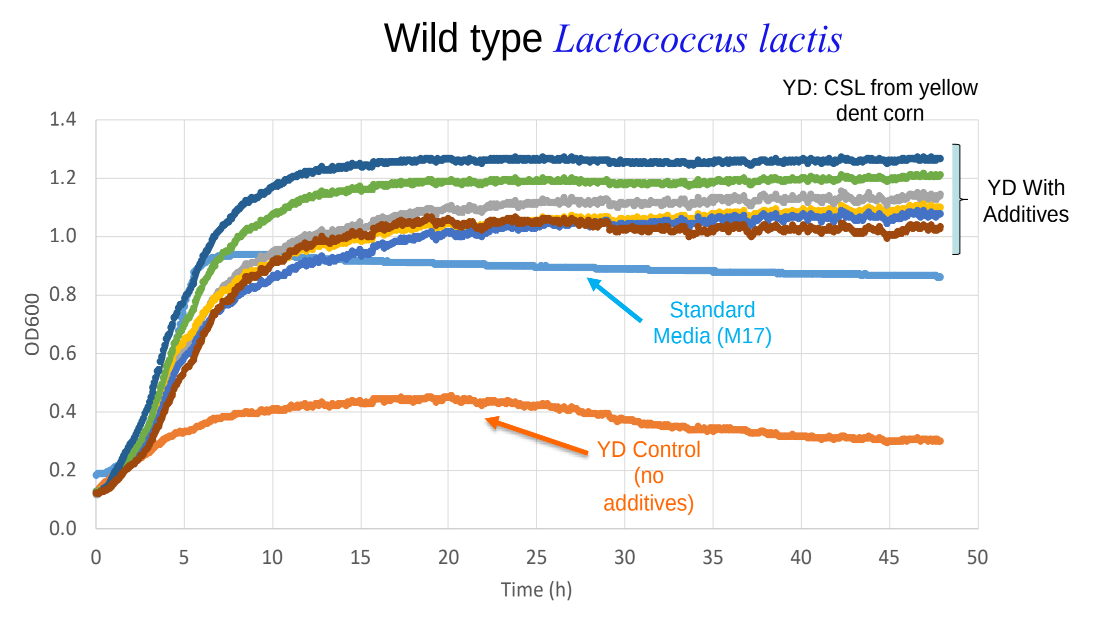
Sampling (0, 6, 12, 24, 48, 72 h)

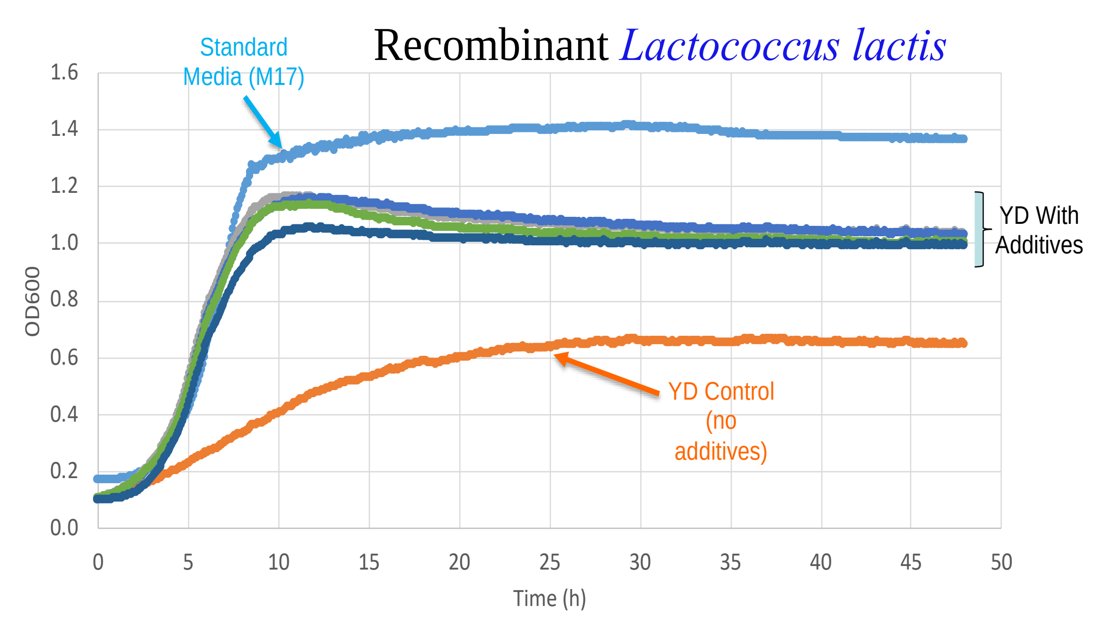
- Microbial viable count
- pH measurement
- Total carbohydrate contents
- Freezing experiment



Freezing experiment

# Additive optimization in 96-well plates




CSL was able to support recombinant cell growth, but at slightly lower levels compared to wild types

# Cell growths in 250-mL flasks

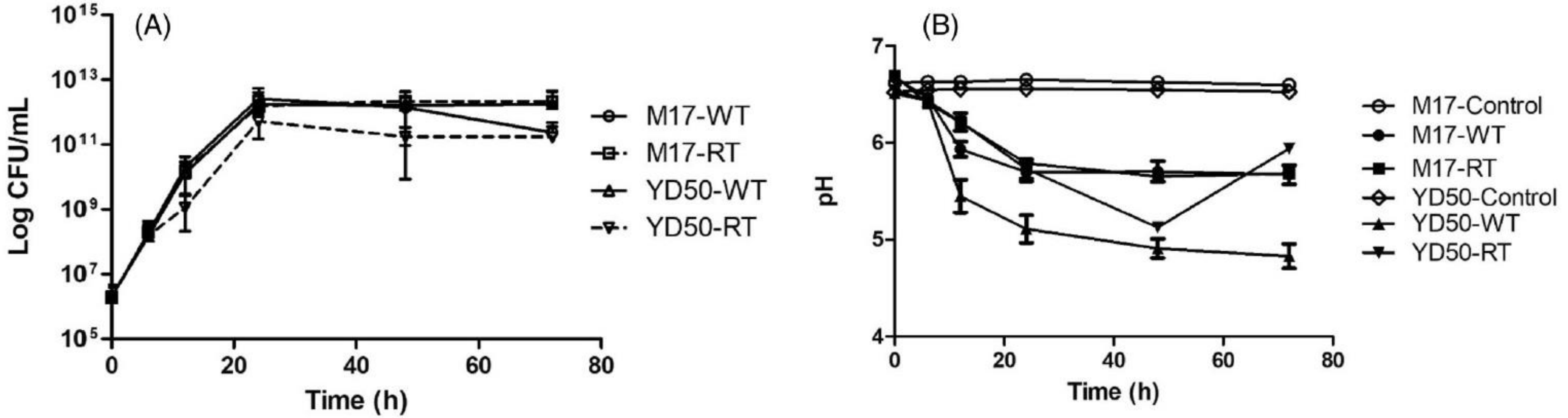




**Figure 4.** Microbial viable population (A) and pH (B) of wild-type (WT) and recombinant-type (RT) *Lactococcus lactis* for M17 media and yellow dent (YD) CSL.

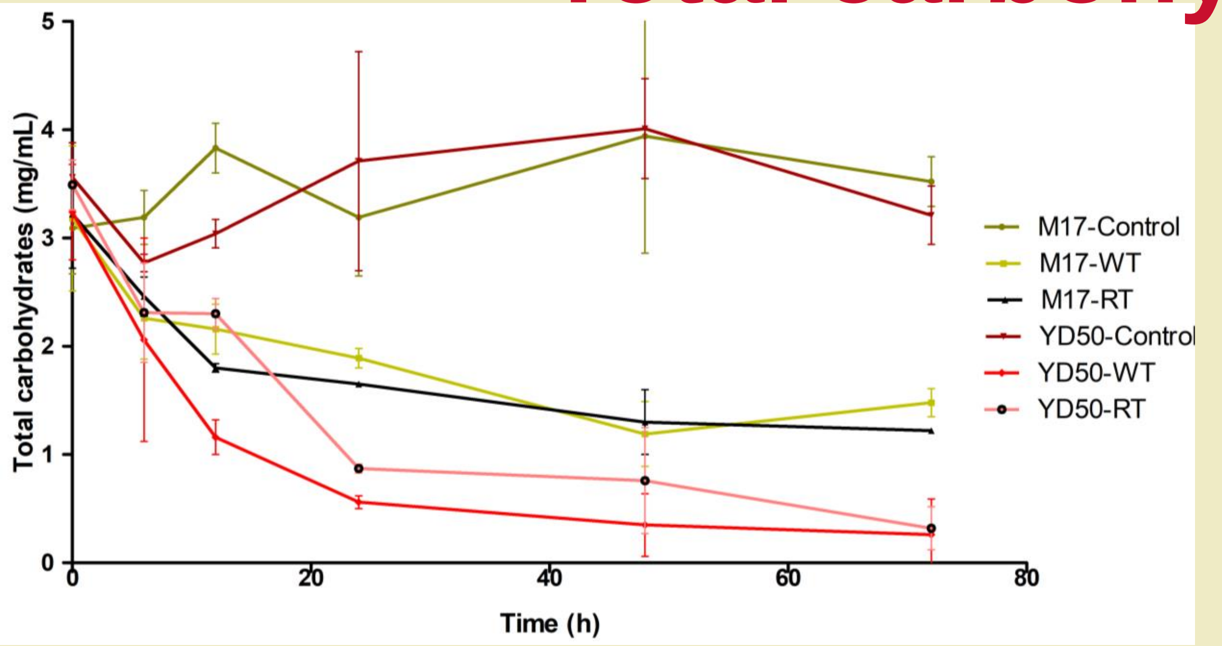
## Optimized corn-steep media supported cell growth very well

# Specific growth rates ($\mu$), and doubling time ($t_d$) of *Lactococcus lactis* on different growth media

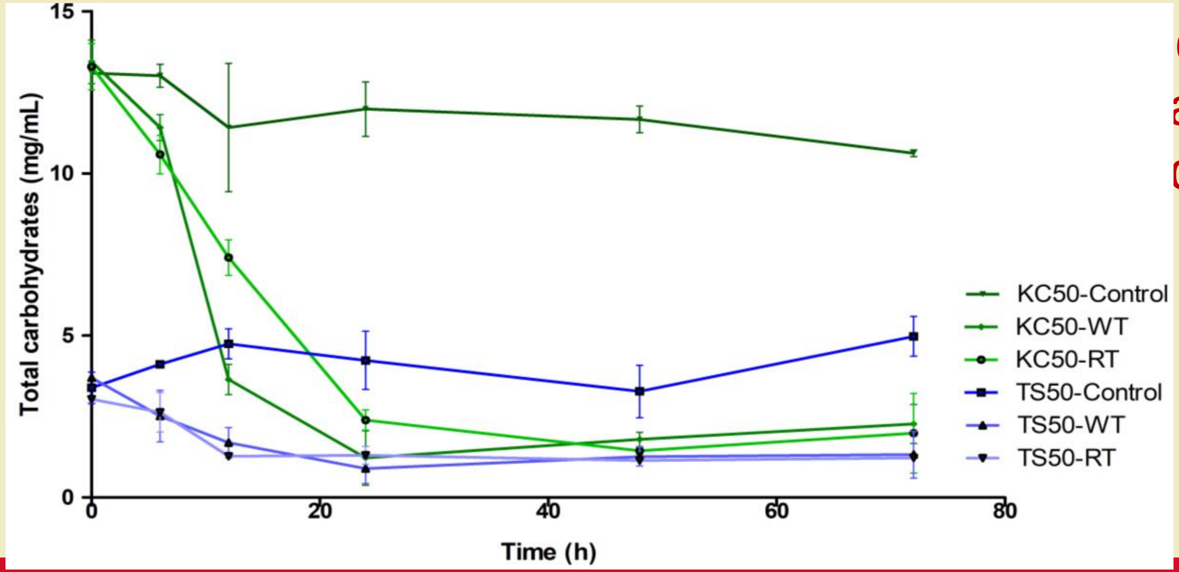
| Media-cells | $\mu$ ($h^{-1}$) | $t_d$ (h) |
|---|---|---|
| M17-WT | 0.52 ± 0.00 [a] | 1.32 ± 0.01 [d] |
| M17-RT | 0.50 ± 0.00 [a] | 1.40 ± 0.00 [d] |
| YD50-WT | 0.52 ± 0.00 [a] | 1.32 ± 0.01 [d] |
| YD50-RT | 0.44 ± 0.02 [b] | 1.58 ± 0.05 [c] |
| KC50-WT | 0.40 ± 0.01 [c] | 1.75 ± 0.04 [b] |
| KC50-RT | 0.39 ± 0.00 [c] | 1.80 ± 0.02 [b] |
| TS50-WT | 0.39 ± 0.01 [c] | 1.77 ± 0.06 [b] |
| TS50-RT | 0.35 ± 0.00 [d] | 2.00 ± 0.02 [a] |

- Compared to the wild type, recombinant type *L. lactis* lower growth rate and longer doubling time.
  - For wild type strain, the highest growth rate (0.52 ± 0.00 $h^{-1}$) and smallest doubling time (1.32 ± 0.01 h) was observed for M17 media.
  - For recombinant strain, the highest growth rate and doubling time were observed for M17 and yellow dent CSL.

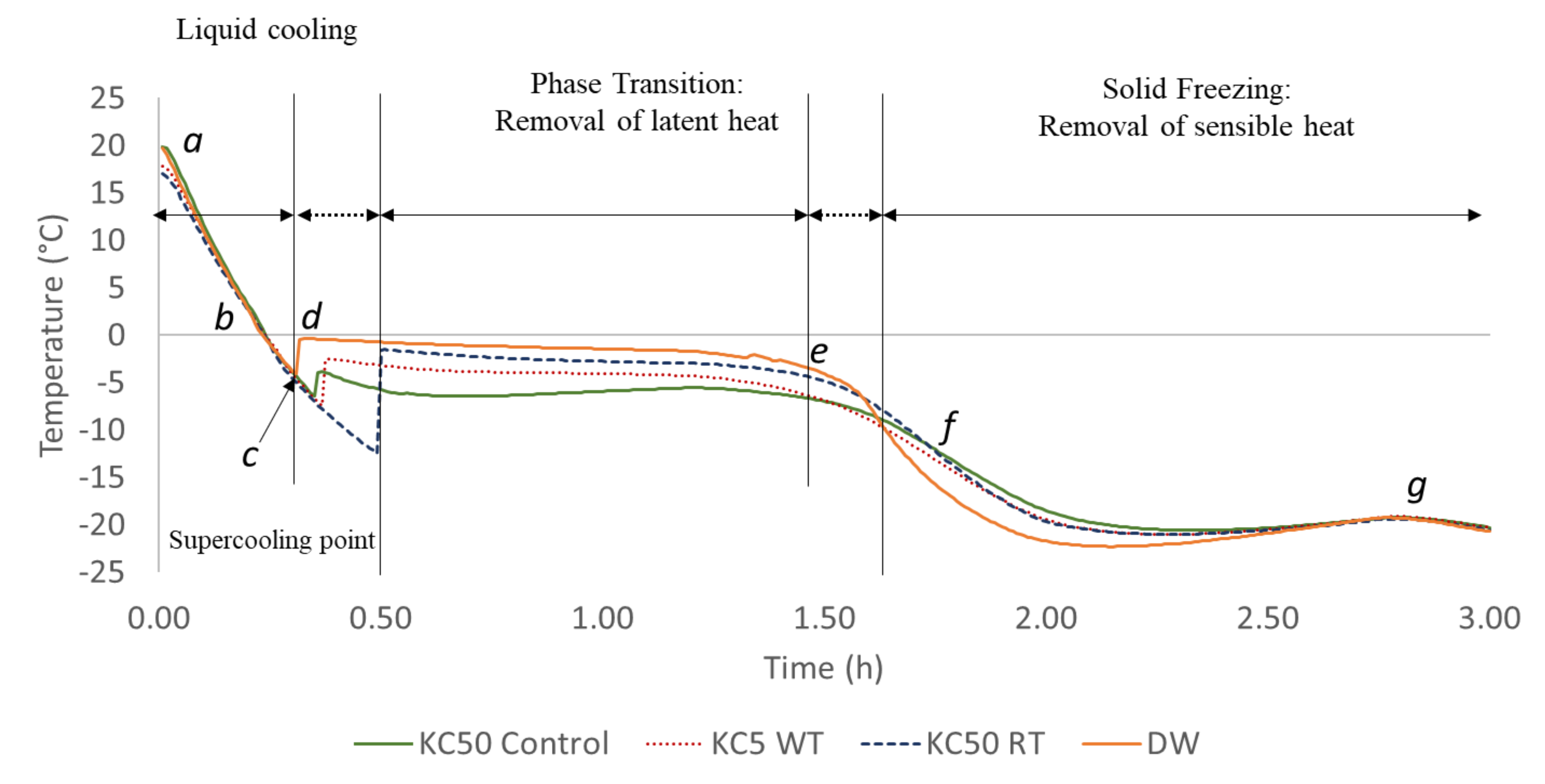
# Total carbohydrate contents




- No changes in controls (without any inoculation).
- Total carbohydrate highest in Industrial CSL (13 mg/mL).
- Both wild type and RT type
d sugars thus decreasing
arbohydrate concentration
during fermentation.



# Freezing experiments with fermentation supernatants




*Representative freezing curves for fermentation supernatants of control (no inoculation), wild, recombinant type Industrial CSL, and distilled water.*

| Media | Supernatant from media | Supercooling temperature (°C) | Supercooling time (h) | Supercooling time (min) | Supercooling time extended (min) |
|---|---|---|---|---|---|
| M17 | Control (No microbes) | -0.93± 0.25 [a] | 0.25 ± 0.01 [b] | 14.7 ± 0.34 | - |
| | Wild-Type | -6.18 ± 4.39 [ab] | 0.47 ± 0.14 [ab] | 28.2 ± 8.50 | 13.2 |
| | Recombinant-Type | -8.56 ± 3.18 [b] | 0.53 ± 0.14 [a] | 31.95 ± 8.23 | 16.8 |
| YD50 | Control (No microbes) | -1.05 ± 1.00 [a] | 0.32 ± 0.04 [a] | 19.05 ± 2.26 | - |
| | Wild-Type | -7.04 ± 3.79 [ab] | 0.36 ± 0.08 [a] | 21.8 ± 4.80 | 2.4 |
| | Recombinant –Type | -7.59 ± 3.57 [b] | 0.43 ± 0.10 [a] | 25.95 ± 5.80 | 6.6 |
| KC50 | Control (No microbes) | -4.70 ± 2.50 [a] | 0.37 ± 0.07 [b] | 22.05 ± 4.33 | - |
| | Wild-Type | -5.76 ± 1.33 [a] | 0. 40 ± 0.08 [b] | 24.0 ± 4.87 | 2 |
| | Recombinant –Type | -11.79 ± 1.44 [b] | 0.55 ± 0.05 [a] | 32.85 ± 3.22 | 11 |
| TS50 | Control (No microbes) | -1.39 ± 0.44 [a] | 0.28 ± 0.02 [b] | 16.8 ± 1.46 | - |
| | Wild-Type | -7.46 ± 4.26 [b] | 0.44 ± 0.11 [ab] | 26.1 ± 6.83 | 9.5 |
| | Recombinant -Type | -9.94 ± 2.70 [b] | 0.57 ± 0.17 [a] | 34.35 ± 10.39 | 18 |

# Summary

- Best combination for CSL based media **DG-5 g/L, T-5 g/L, A.A-0.5 g/L, and zinc chloride (4 ppm).**

- Compared to the wild type, recombinant type *L. lactis* had lower growth rate and longer doubling time.

- For both wild and recombinant type strain: M17 showed the highest growth rate and lowest doubling time followed by yellow dent CSL.

- Initial freezing time and temperature were significantly extended by recombinant strain for all the corn coproduct-based media.

# Some discussion points:

1. **Bioprocessing outlook in Nepal**
   - Related engineering disciplines and universities producing trained manpower
   - Industries: how developed are they to take in graduates?
2. **What/ How we can contribute to Bioprocessing?**