Converting Agricultural Waste into Sustainable and Value-added Products for Efficient Material and Chemical (Succinic Acid) Production

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Agricultural waste and residue management for a circular bio-economy: Shared EU and China impact-oriented solutions
Section A2. Challenges and Perspectives of Up-Cycling Agricultural Wastes and Residues into Sustainable Bio-Products using Eco-Friendly Technologies
October 22, 2018 at 17:30 - Hubei Hotel, Haidian District, Zhongguan South Street, No. 36, Beijing, China

Overview

- Introduction of NOAW's WP4 rationale and objectives
- Development of agro-waste bio-refinery concepts : two examples
  - Lignocellulosic-based biocomposites for food packaging
  - Succinic acid production using metabolic evolutionary
- Conclusions and acknowledgements

NOAW and WP4 context

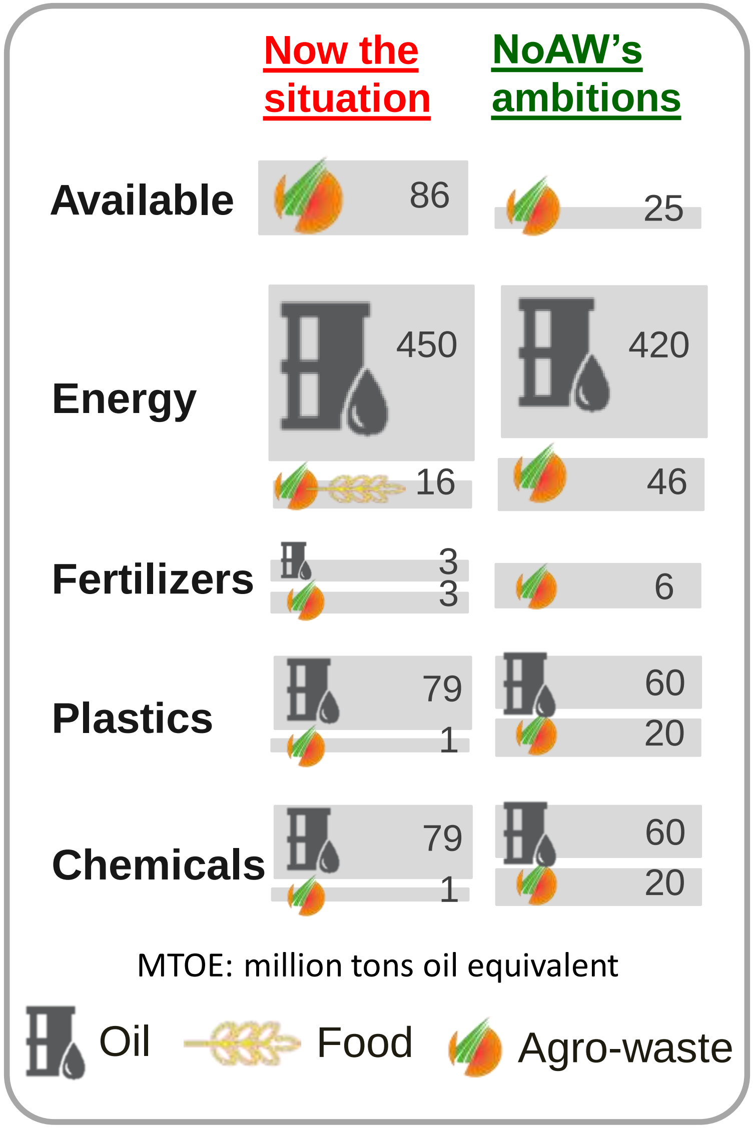
Towards real recyclability

by developing a **circular economy approach** applicable to agro-wastes on a territorial and seasonal basis,

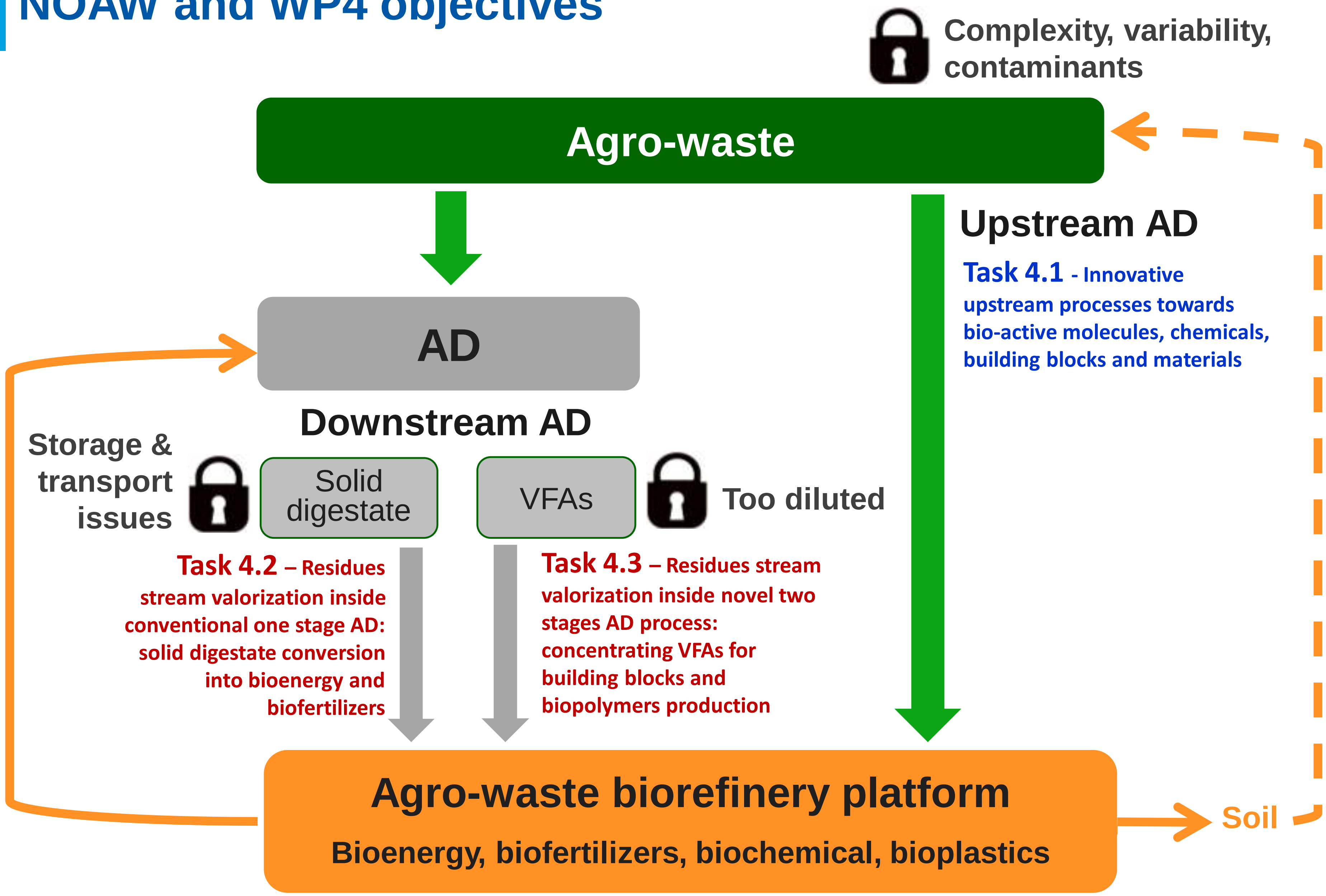
by developing **added-value bioproducts** without penalising side-effects on soils, water and air quality

NOAW and WP4 objectives

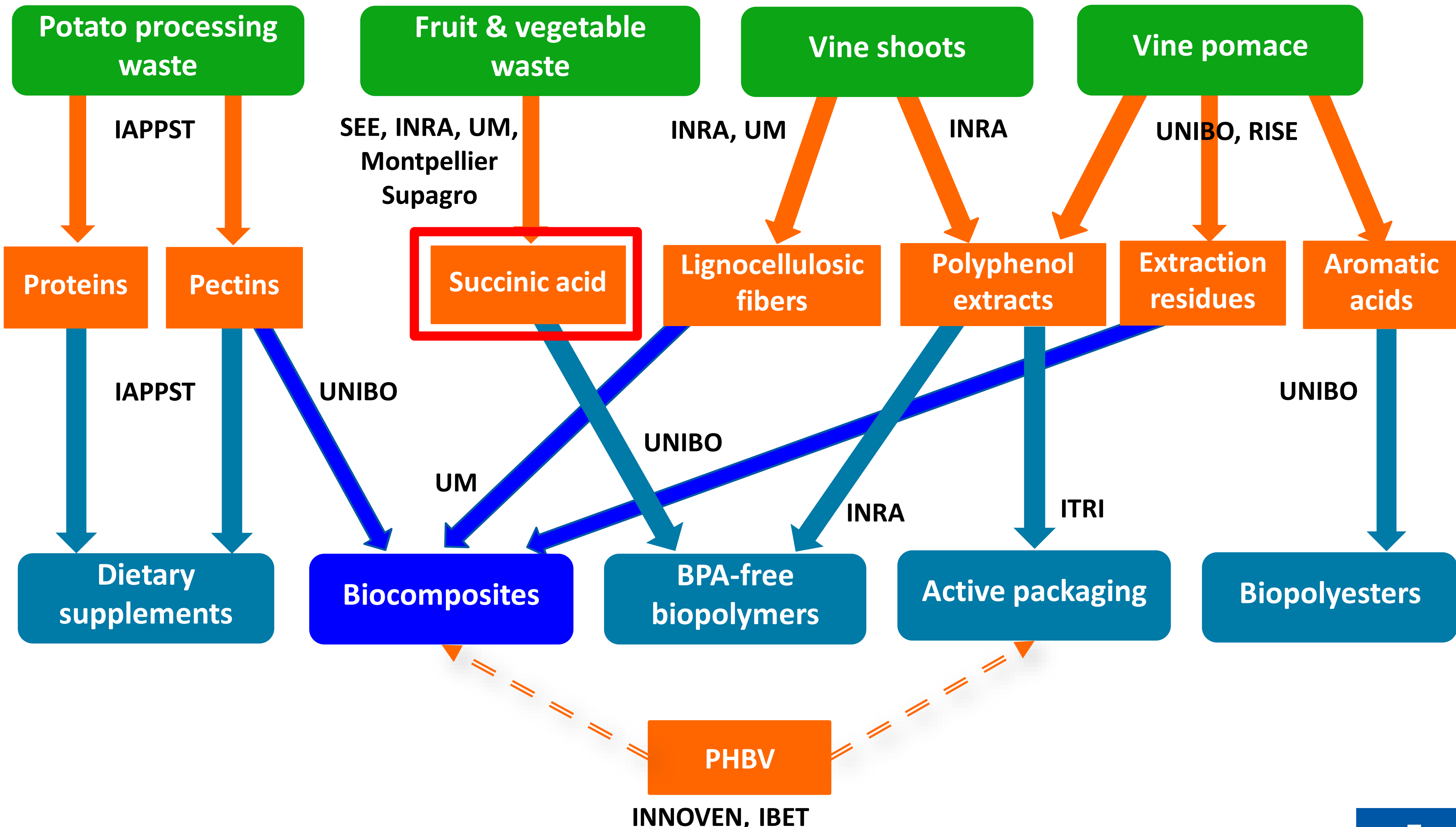
- To up-cycle more than 75% of agro-waste
- into **bioenergy**, **biofertilizers**, **biochemicals** and biodegradable innocuous **bioplastics**,
  - to substitute non-food crops uses
  - to contribute to oil resources saving
  - to increase renewable energy
  - to eradicate food packaging plastics accumulation
  - to ensure nutrients back to the soil
  - to reduce the negative impact of inappropriate agro-waste management
- using eco-efficient technologies to limit carbon emission.



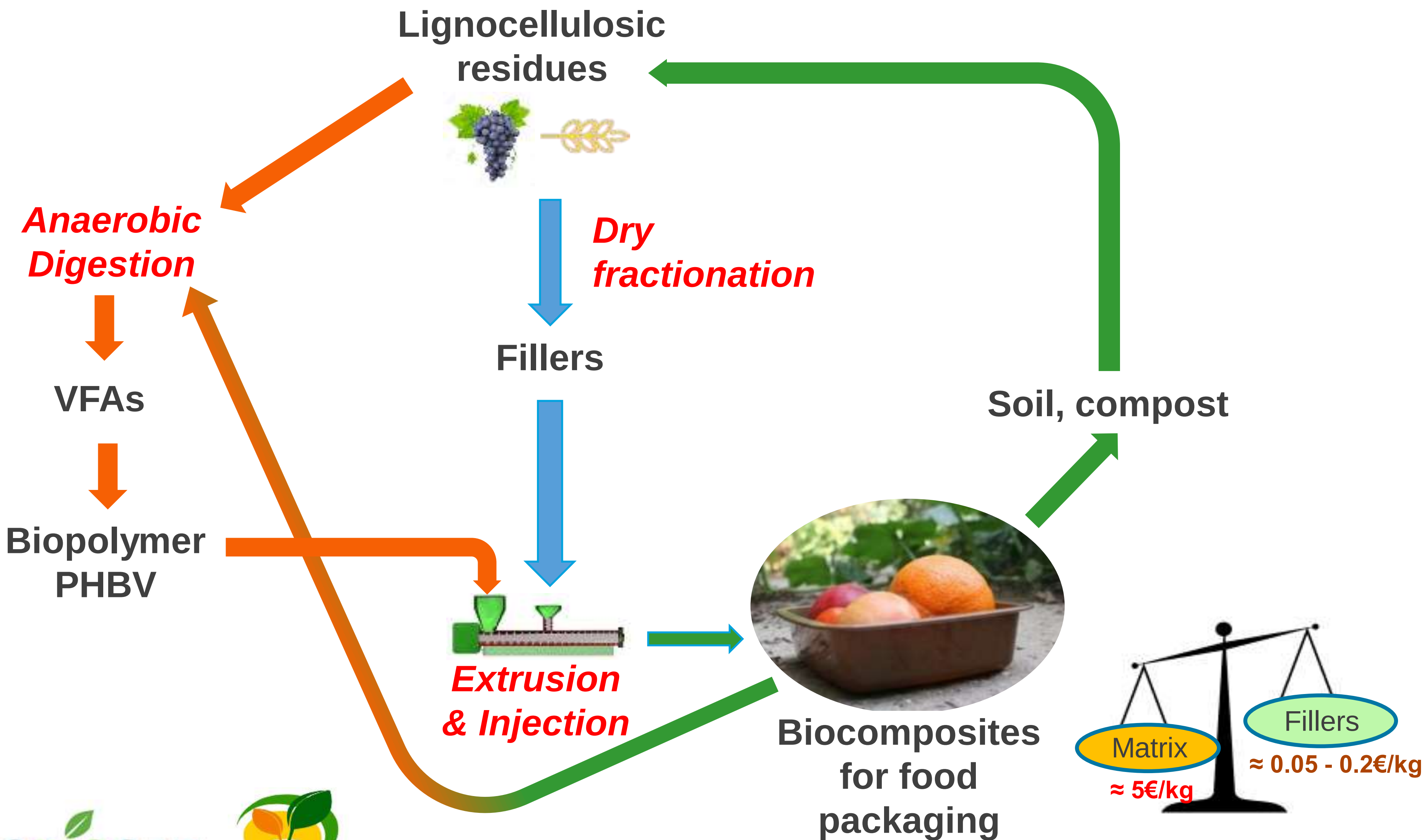
NOAW and WP4 objectives



Task 4.1. Different value chains according to the initial biomass (upstream AD)

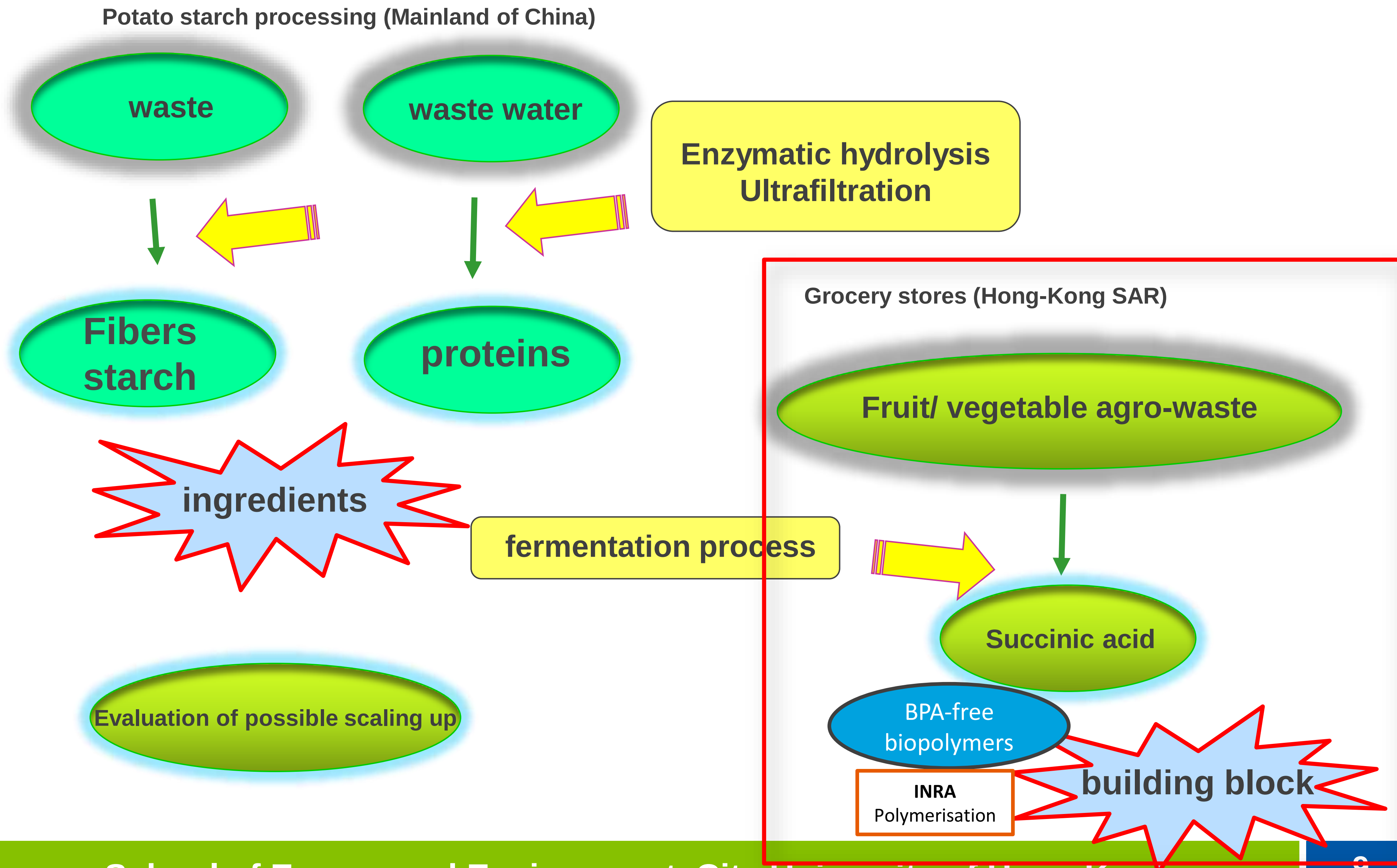


Example 1 : PHBV/lignocellulosic fibers biocomposites for food packaging

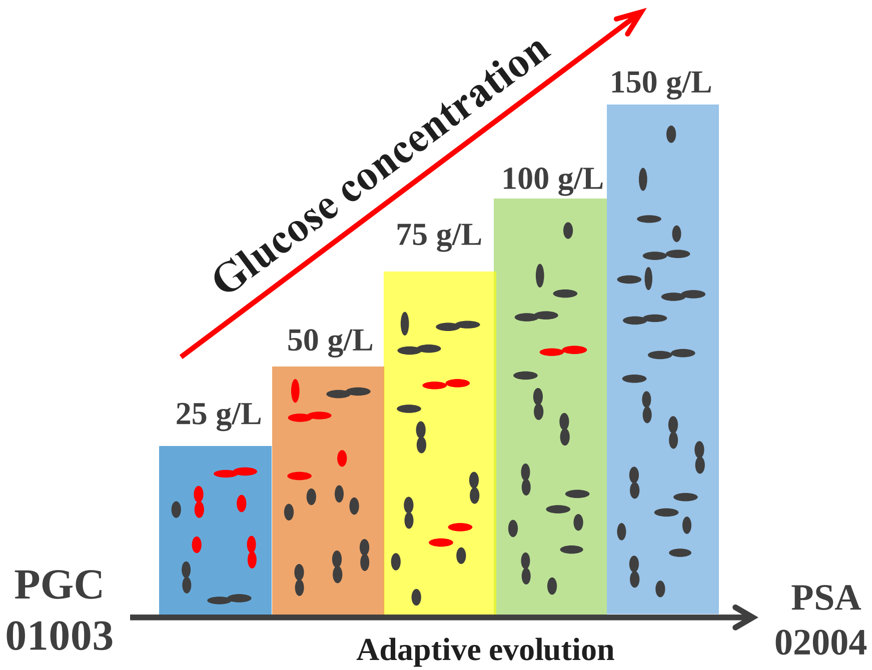


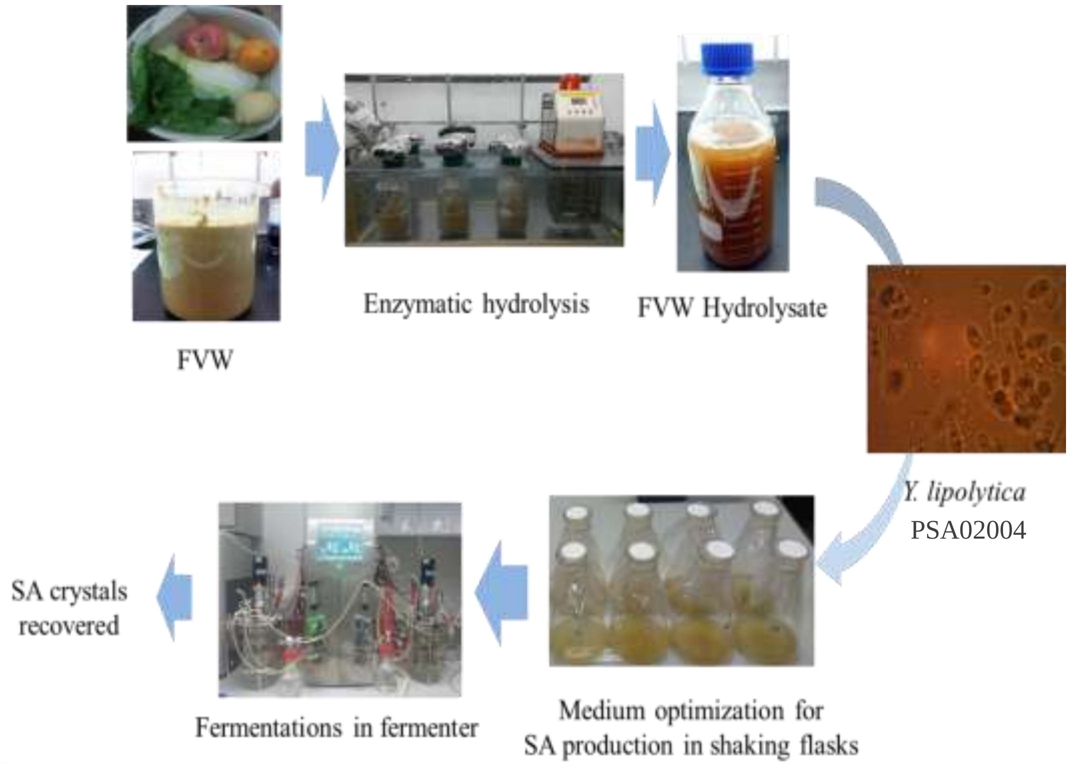
ECOBIOCAP NCAW

Example 2 : Succinic acid production using agricultural waste



SA production using fruit and vegetable waste as substrate



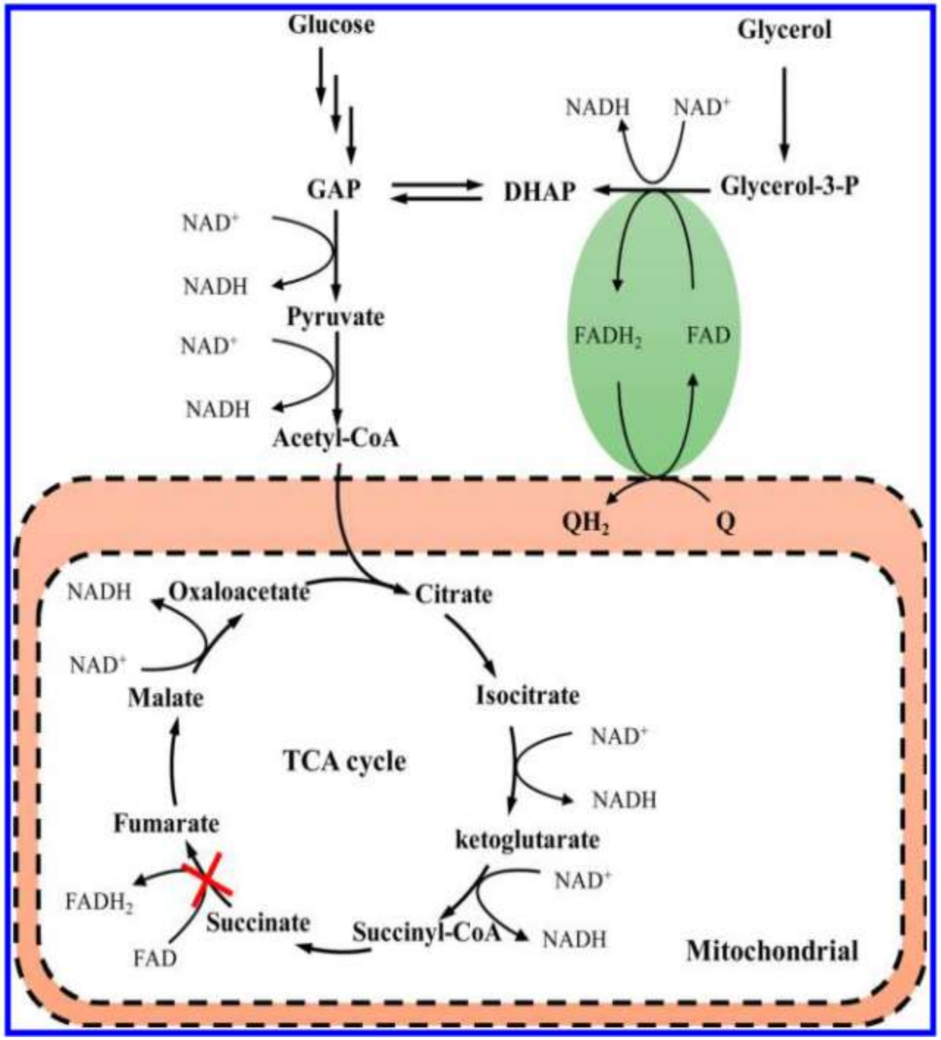


Adaptive evolution of PGC01003 (glycerol) to PSA02004 (glucose)

SA production from fruit & vegetable waste hydrolysate

Publication: Yang, X., Wang, H., Li, C., **Lin, C.S.K.** 2017. Restoring of glucose metabolism of an engineered *Yarrowia lipolytic*a for succinic acid production *via* a simple and efficient adaptive evolution strategy. *Journal of Agricultural and Food Chemistry*, 65, 4133-4139.

PSA02004



1. Parent strain of PSA02004: PGC01003

2. Substrate: glucose at pH 6.0.

3. Pathway: the same to PGC01003, but the pathway from glucose might be activated.

4. Related papers:

1) Yang, X., Wang, H., Li, C., Lin, C.S.K. 2017. Restoring of Glucose Metabolism of Engineered *Yarrowia lipolytica* for Succinic Acid Production via a Simple and Efficient Adaptive Evolution Strategy. J Agric Food Chem, 65(20), 4133-4139.
2) Li, C., Yang, X., Gao, S., Chuh, A.H., Lin, C.S.K. 2018. Hydrolysis of fruit and vegetable waste for efficient succinic acid production with engineered *Yarrowia lipolytica*. Journal of Cleaner Production(179), 151-159.

Glucose production using fruit and vegetable as substrate

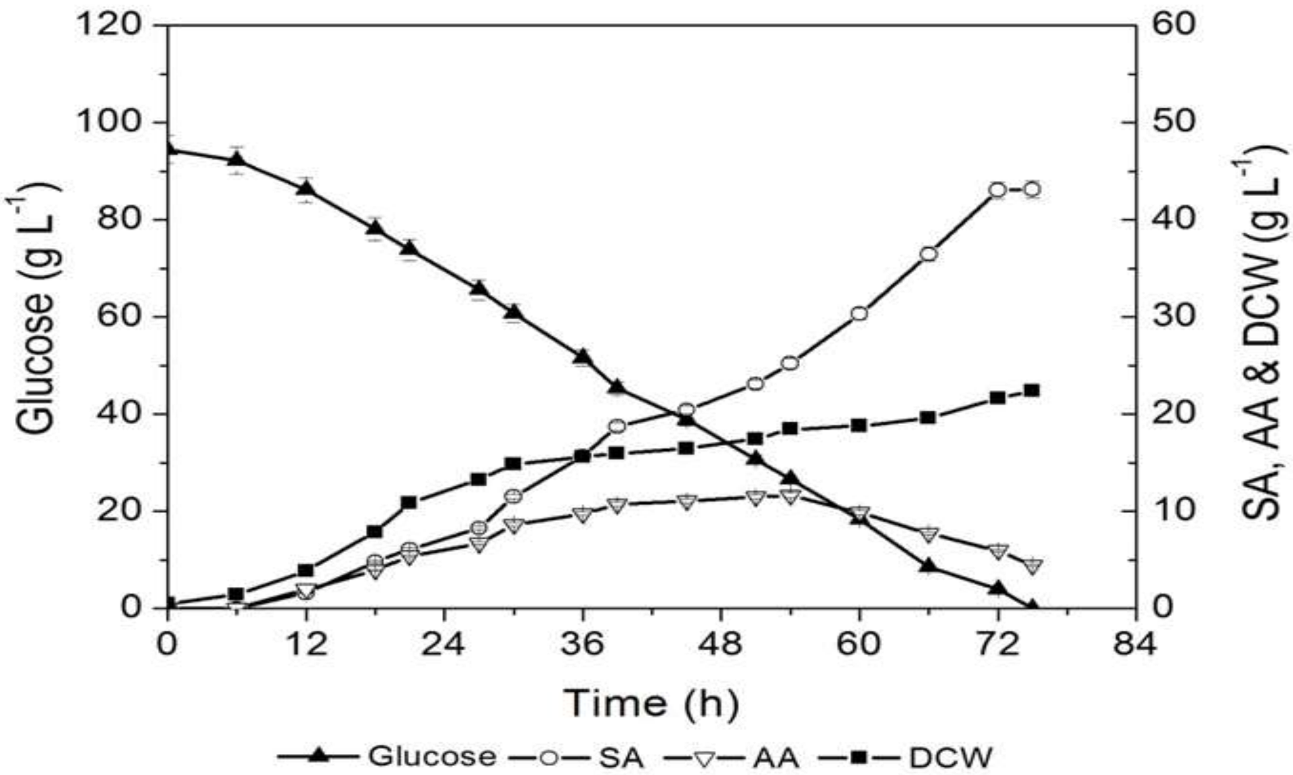
Glucose production from enzymatic hydrolysis with various dosages of enzyme

Enzyme Optimized	Dosage of other enzymes	Dosage (%, w/w FVW)	Initial glucose (g L^{-1})	Glucose produced (g L^{-1})	Yield (g g^{-1})
Glucoamylase	Cellulase (1%) Hemicellulase (0.5%) Pectinase (0.5%)	0.25	10.3±0.3	36.6±0.4	0.38±0.01
		0.5	10.0±0.3	39.5±0.5	0.41±0.01
		1.0	9.9±0.4	41.0±0.5	0.42±0.01
		2.0	10.9±0.5	43.8±0.2	0.45±0.01
		3.0	11.6±0.5	43.9±0.2	0.45±0.01
		4.0	12.5±0.5	43.3±0.5	0.44±0.01
Cellulase	Glucoamylase (2%) Hemicellulase (0.5%) Pectinase (0.5%)	0.25	12.1±0.5	36.1±0.5	0.37±0.01
		0.5	12.1±0.5	35.5±0.5	0.37±0.01
		1.0	12.8±0.5	43.1±0.5	0.45±0.01
		2.0	11.7±0.5	42.8±0.5	0.44±0.01
		3.0	11.1±0.5	43.2±0.5	0.45±0.01
		4.0	13.1±0.5	42.8±0.5	0.44±0.01
Hemicellulase	Glucoamylase (2%) Cellulase (1%) Pectinase (0.5%)	0.25	12.4±0.4	36.9±0.5	0.38±0.01
		0.5	12.5±0.5	42.4±0.5	0.44±0.01
		1.0	12.2±0.2	41.7±0.5	0.43±0.01
		2.0	12.4±0.4	44.3±0.5	0.46±0.01
		3.0	12.3±0.3	44.2±0.5	0.46±0.01

- **Glucose (44.3 g/L)** was produced from FVW under the optimized conditions.

2% glucoamylase, 1% cellulase, 2% hemi-cellulase and 0.25% pectinase at pH 5.0 and 55 °C

SA production using fruit and vegetable waste as substrate



SA production under the optimized medium

SA titer at 43.1 g/L with a yield at 0.45 g/g was achieved from FVW hydrolysate.

Publication: Li, C., Yang, X., Gao, S., Lin, C.S.K. 2018. Hydrolysis of fruit and vegetable waste for efficient succinic acid production with engineered *Yarrowia lipolytica*. *Journal of Cleaner Production*, 179, 151-159.

Fermentation strategies for improved SA production

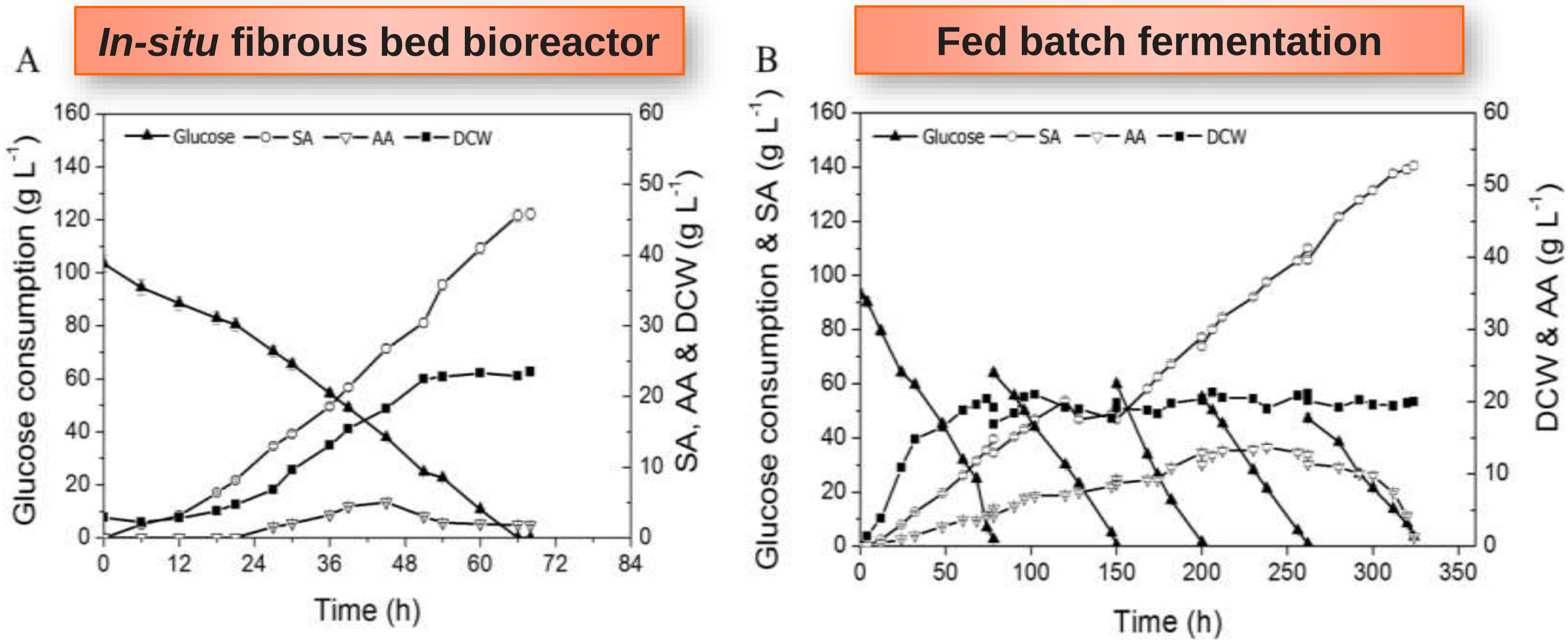


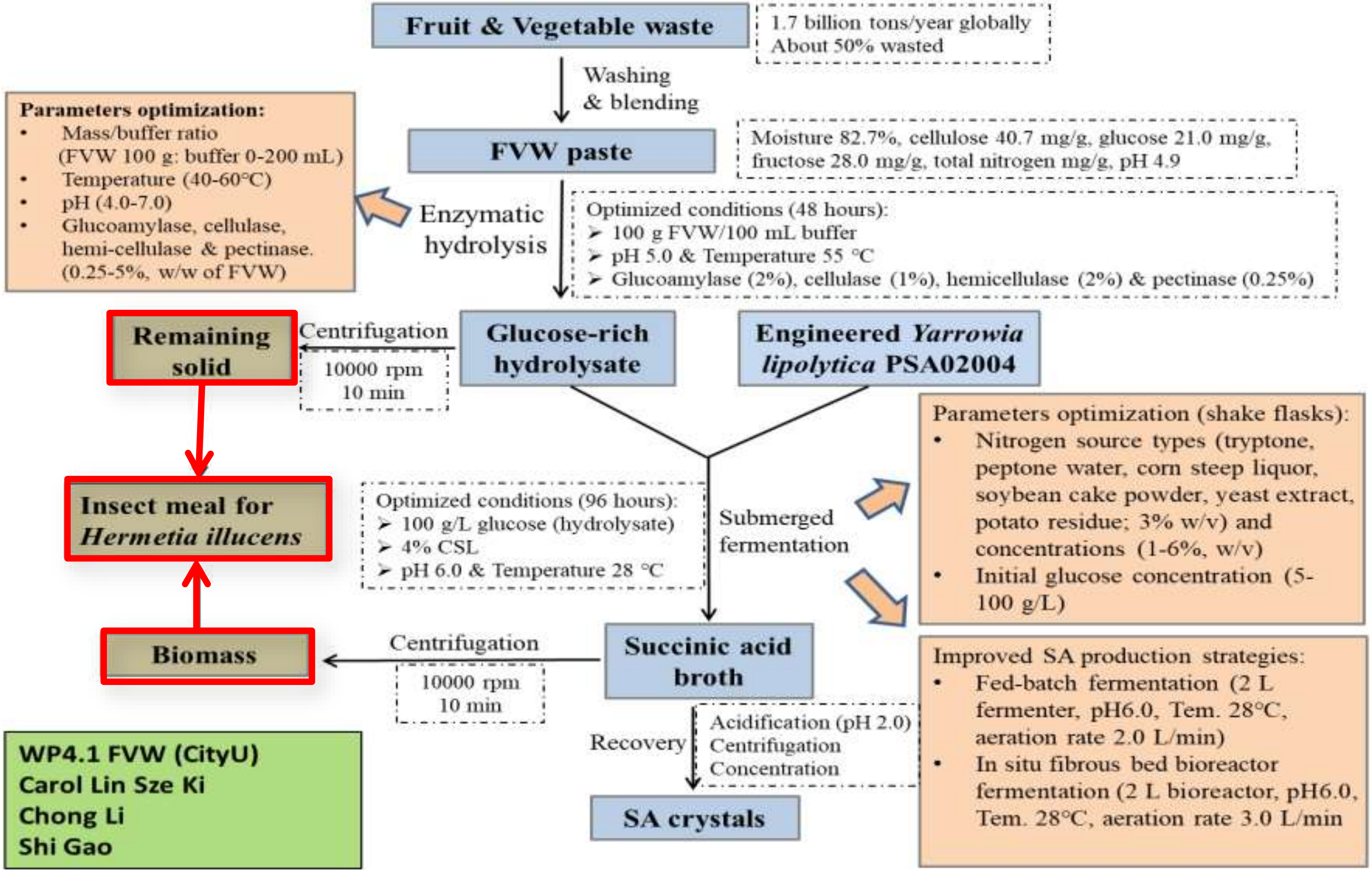
Table 6
Summary of SA production by different fermentation modes.

Fermentation modes	Time (h)	SA titer ($g\ L^{-1}$)	SA productivity ($g\ L^{-1}\ h^{-1}$)	SA yield ($g\ g^{-1}$)	DCW ($g\ L^{-1}$)	Biomass holdup (g)[a]
Batch	75 ± 1	43.1 ± 1.0	0.57 ± 0.02	0.46 ± 0.01	22.4 ± 2.0	N/A [b]
*is*FBB	66 ± 1	45.6 ± 1.0	0.69 ± 0.02	0.46 ± 0.01	23.3 ± 2.0	9.5 ± 1.0
Fed batch	324 ± 1	140.6 ± 2.0	0.44 ± 0.01	0.47 ± 0.01	20.4 ± 3.0	N/A

[a] Biomass hold up refers to the cells immobilized to the cotton towel in *is*FBB.
[b] Not applicable.

Li, C., Yang, X., Gao, S., Chuh, A.H., Lin, C.S.K. 2018. Hydrolysis of fruit and vegetable waste for efficient succinic acid production with engineered *Yarrowia lipolytica. Journal of Cleaner Production,* ***179, 151-159.***

Fruit & vegetable waste into valuable chemicals



WP4.1 FVW (CityU)
Carol Lin Sze Ki
Chong Li
Shi Gao

SA production at low pH

Why low pH?

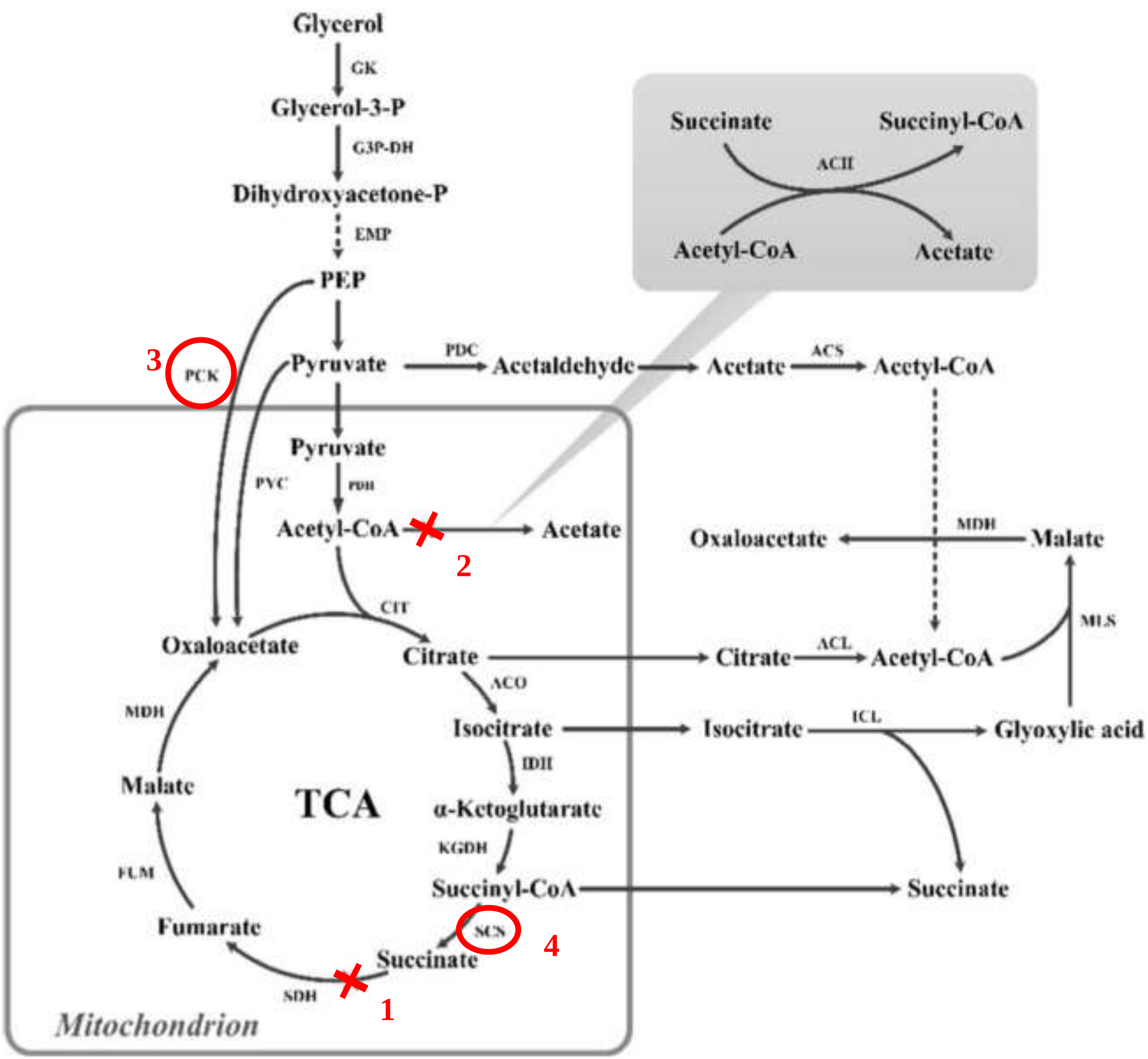
- Acidification of succinate from fermentation broth (pH 6.0) to recover succinic acid crystals (pH 2.0) takes 60-70% of the total production cost [1-2].

Why *Y. lipolytica*?

- Bacteria considered as feasible succinate producers are unable to grow effectively at low pH values [1-4].
- SA production from glycerol by *Y. lipolytica* PGC202 has been demonstrated by our collaborator [4].

1. Cok, B., Tsiropoulos, I., Roes, A.L., Patel, M.K. 2014.. Biofuels, Bioproducts and Biorefining, 8(1), 16-29.
2. Zeikus, J.G., Jain, M.K., Elankovan, P., 1999. Applied Microbiology and Biotechnology. 51, 545-552.
3. Andersson, C., Helmerius, J., Hodge, D., Berglund, K.A., Rova, U., 2009. Biotechnology Progress. 25, 116-123.
4. Cui, Z., Gao, C., Li, J., Hou, J., Lin, C.S.K., Qi, Q. 2017. Metabolic Engineering, 42, 126-133.

PGC202



Parent strain: should be modified from PGC01003.

Substrate: Glycerol at pH without control (pH 2-3)

Pathway:

1. Inactivation of succinate dehydrogenase (SDH): produce high SA at pH 6.0 but more acetic acid. **Po1fe (PGC01003).** Acetic acid may negatively influence cell growth and SA production, which might be the reason that *Y. lipolytica* can not produce SA at low pH.

2. Deleting YlACH (acetyl-CoA hydrolase): AA decreased from 7.5 g/L to 0.2 g/L. SA production and cell growth were improved (27.3% in SA titer). But too much pyruvate accumulation (0.4-6.0 g/L).

3. Overexpression of ScPCK (phosphoenolpyruvate carboxykinase from *Saccharomyces cerevisiae*): eliminating the accumulation of pyruvate, and increased SA production by 150.2%.

4. Further improve SA production by overexpressing YlSCS (succinyl-CoA synthase): 24% higher in SA titer than Step 3.

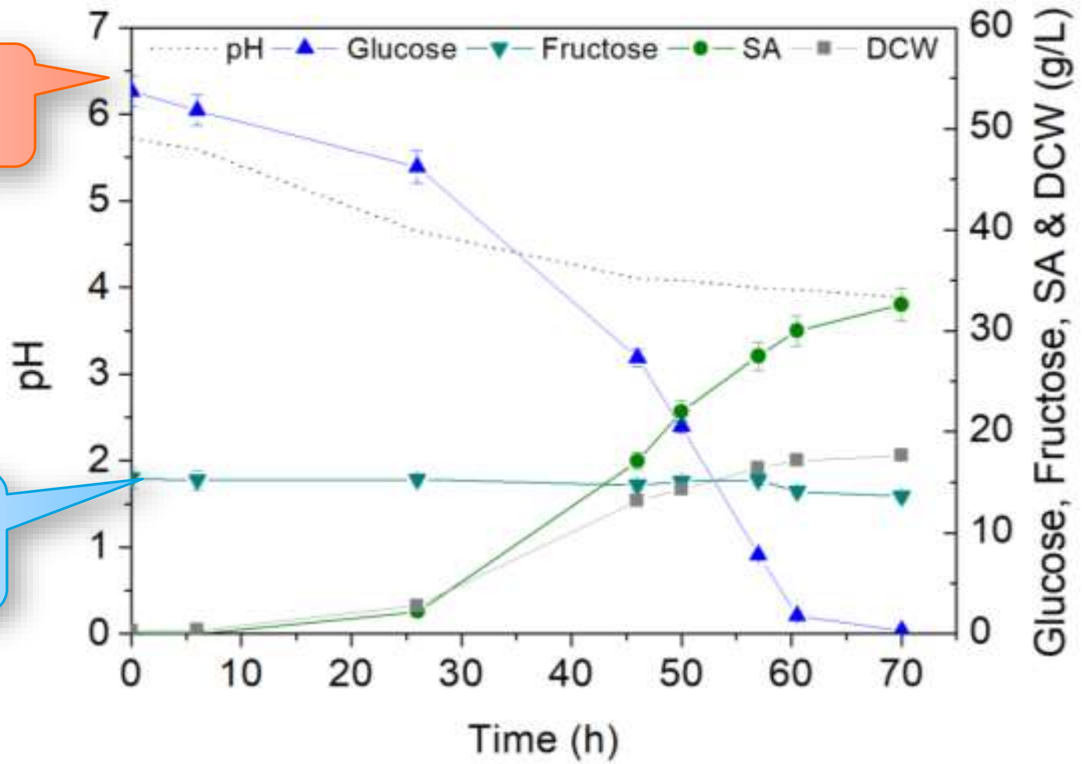
Related Paper:

Cui, Z., Gao, C., Li, J., Hou, J., Lin, C.S.K., Qi, Q. 2017. Engineering of unconventional yeast *Yarrowia lipolytica* for efficient succinic acid production from glycerol at low pH. Metab Eng, 42, 126-133.

SA production from fruit & vegetable waste by PGC202

56.7 g/L glucose

16.4 g/L fructose



- Fermentative SA production was performed without pH control by ***Y. lipolytica*** **PGC202**. **(pH decreased from 5.7 to 3.9)**
- **32.6 g/L SA with a yield of 0.61 g/g** was produced from FVW hydrolysate.
- DCW was 17.6 g/L
- **Fructose kept stable at around 15 g/L before the depletion of glucose**.

Summary of research work

Novelties	Conclusions
▪ **Feasibility of using fruit and vegetable waste hydrolysate for SA production by *Y. lipolytica* was demonstrated.** ▪ **Application of metabolic engineering to obtain *Y. lipolytica* for bio-synthesis of SA from glucose at low pH was demonstrated.**	• SA titer at **43.1 g/L** was obtained from fermentation of fruit and vegetable hydrolysate. • **Acetate** was found to be the reason for the inhibition of *Y. lipolytica* at **low pH.** • *Y. lipolytica* has **extensive substrate adaptability**. • **Highest SA yield of 0.61 g/g** was achieved using **fruit and vegetable waste hydrolysate** by **PGC202 in *in-situ* FBB without pH control**

Acknowledgements

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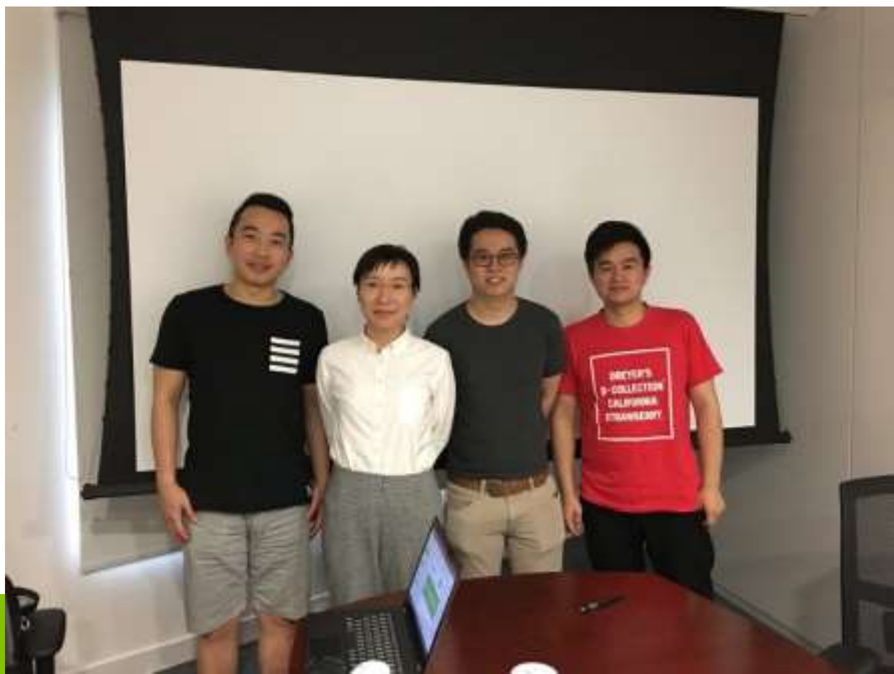
Group members: Dr. Chong Li, Mr. S. Gao, Dr. X.F., Yang, Mr. H.M., Wang, Mr. Khai Lun Ong, Ms. Xiaotong Li, Mr. Harmony Chuh

Collaborators: Prof. Qingsheng Qi & Dr. Cuijuan Gao from Shandong University (*Y. lipolytica*)



Thank you!