Value Addition to Agro-Industrial By-products

INTERNATIONAL WORKSHOP ON FOOD LOSS AND WASTE PREVENTION IN SOUTH ASIAN REGION
INDIAN COUNCIL OF AGRICULTURAL RESEARCH (ICAR) &
THÜNEN INSTITUTE, GERMANY

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Large amount of Agro-wastes - Disposal is an increasing concern

- **India (with 17 % of the world population)** produces large volumes of **rice and wheat** for domestic consumption and export

- According to the Indian Ministry of New and Renewable Energy (MNRE), India generates an average 500 Million tons (Mt) of crop residue per year out of which **15-20 Mt is burnt each year**

The burning of crop residues causes numerous environmental issues :

- **Emission of greenhouse gases - contributes to the global warming**
- **Increased levels of particulate matter (PM)**
- **Smog that cause health hazards**
- **Deterioration of soil fertility**

Agro-waste generation in India comparison to some Asian countries

Country	Agro-waste generated (Mt/year)
India	500
Bangladesh	72
Indonesia	55
Myanmar	19

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6427124/

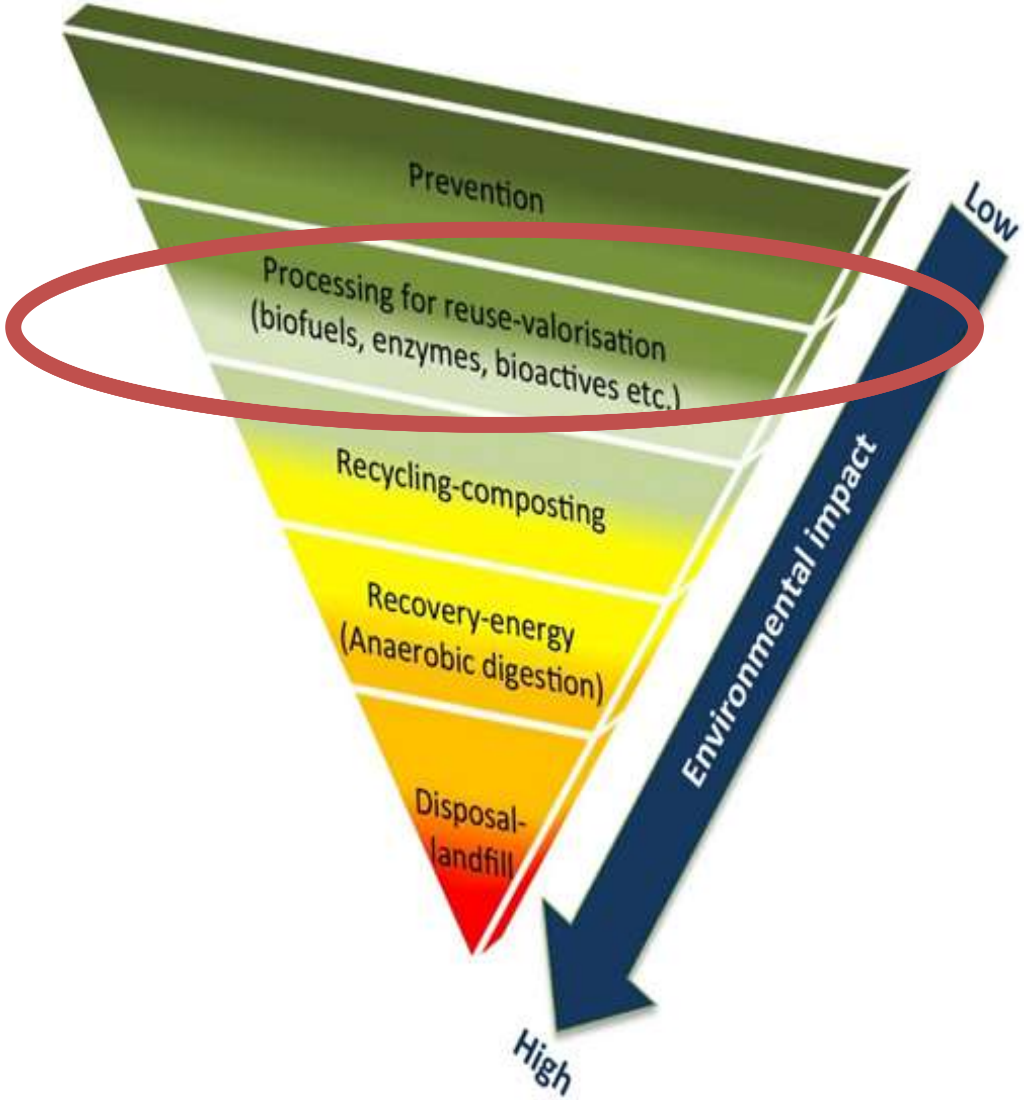
Production level of major crops in India

Crop	Estimated Production (Mt)
Rice	126
Wheat	94
Sugarcane	361
Cotton	35

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6427124/

Waste Framework Directive (WFD) for waste management

- ✓ Disposal in form of incineration or landfill is the least favoured stage of waste management.
- ✓ Valorization of waste needs to be actively pursued: waste to wealth green technologies.



Adopted from *Trends Biotechnol.* 2016;34(1):58-69

Biomass valorization into value-added products

Food waste

Agricultural residues

Industrial waste

Forest residues

Pre-treatment → **Hydrolysis** → **Fermentation**

Solid State Fermentation

- *Types*

Submerged Fermentation

Biofuels:
Bio-oil
Biodiesel
Bioethanol
Biobutanol
Syngas

Biochemicals:
Propylene
Ethylene
Succinic acid
Fumaric acid
Maleic acid
Malic acid
Gluconic acid
Itaconic acid
Citric acid
Levulinic acid
Lactic acid

Some potential waste feedstocks for fermentation

Food waste	Agricultural waste	Industrial waste

Peels of fruits and vegetables

Leftover food

husk/straw/bagasse

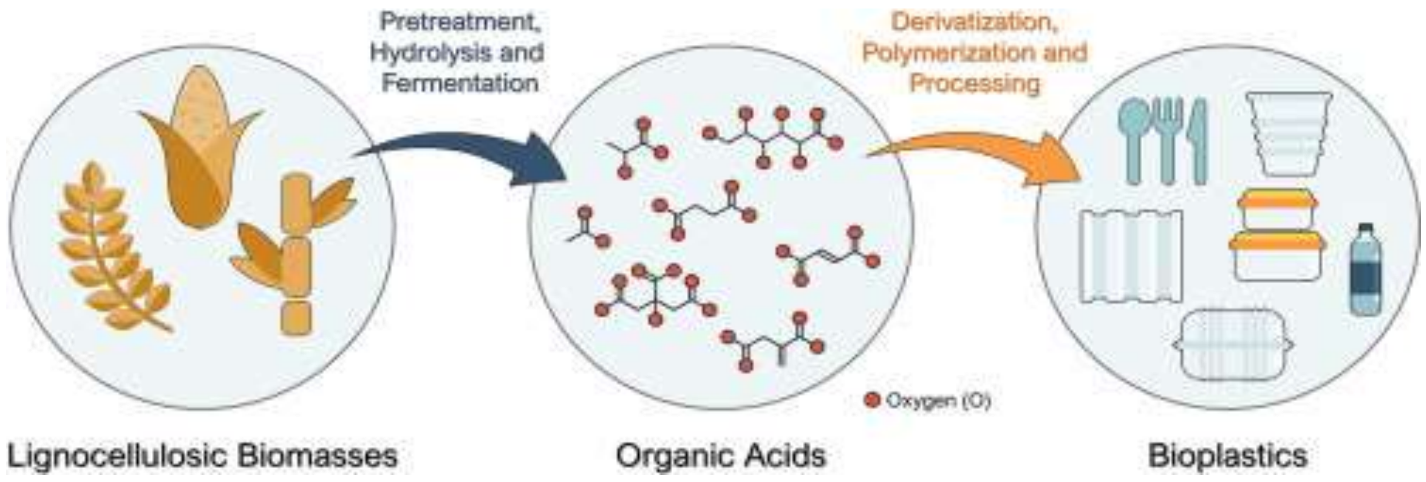
Corn cobs

Waste paper sludge

Waste cotton fibers

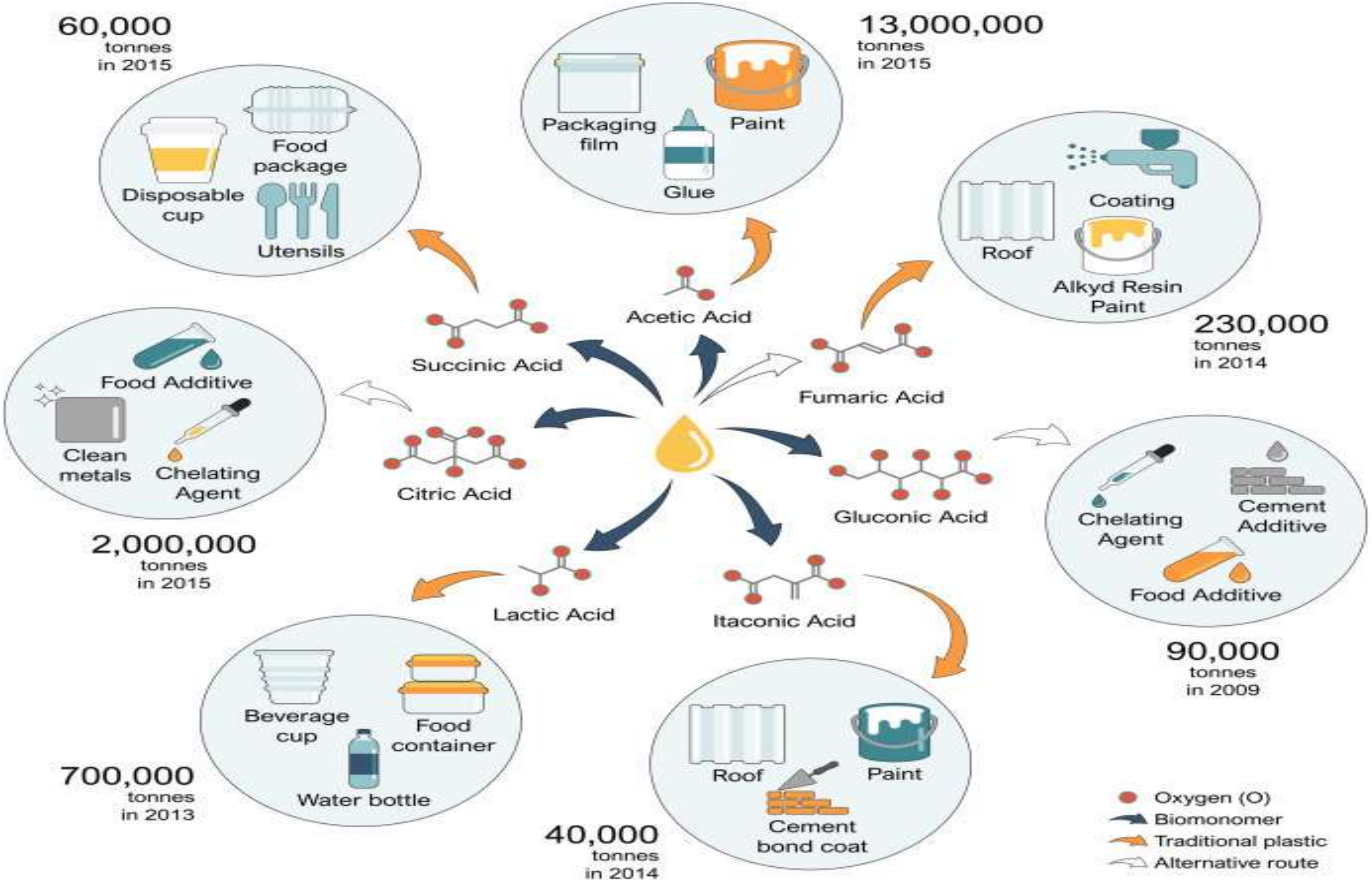
These are mostly lignocellulosics

Bio-based production of platform chemicals



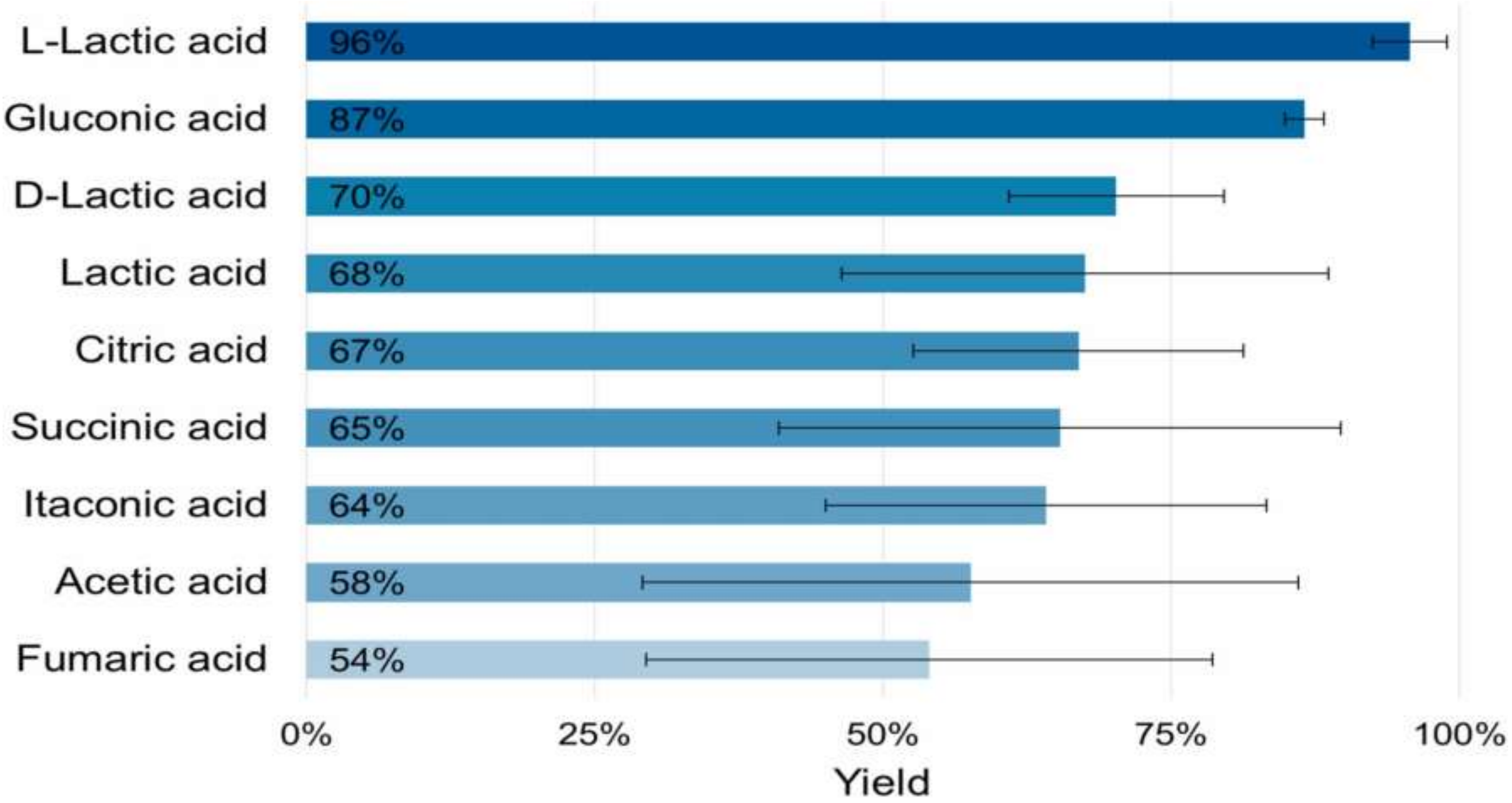
- Platform chemicals are building-block chemicals for producing **various other higher value-added products**.
- These chemicals can be produced in biorefineries using specialized microorganisms/ (currently through petroleum refineries- non eco-friendly and exhausting resources)
- Organic acids can be converted into various higher value-added products with different commercial applications.
- They are an important platform for the chemical industry with high economic potential.
- Microbial factories can utilize inexpensive materials, viz. renewable feedstocks and organic wastes as substrates in the biorefineries, making the production of value-added chemicals more **cost-effective** and **eco-friendly**

Applications and global market potential of platform chemicals



Júnior et al., 2021. *Biomass and Bioenergy*, *149*, 106092.

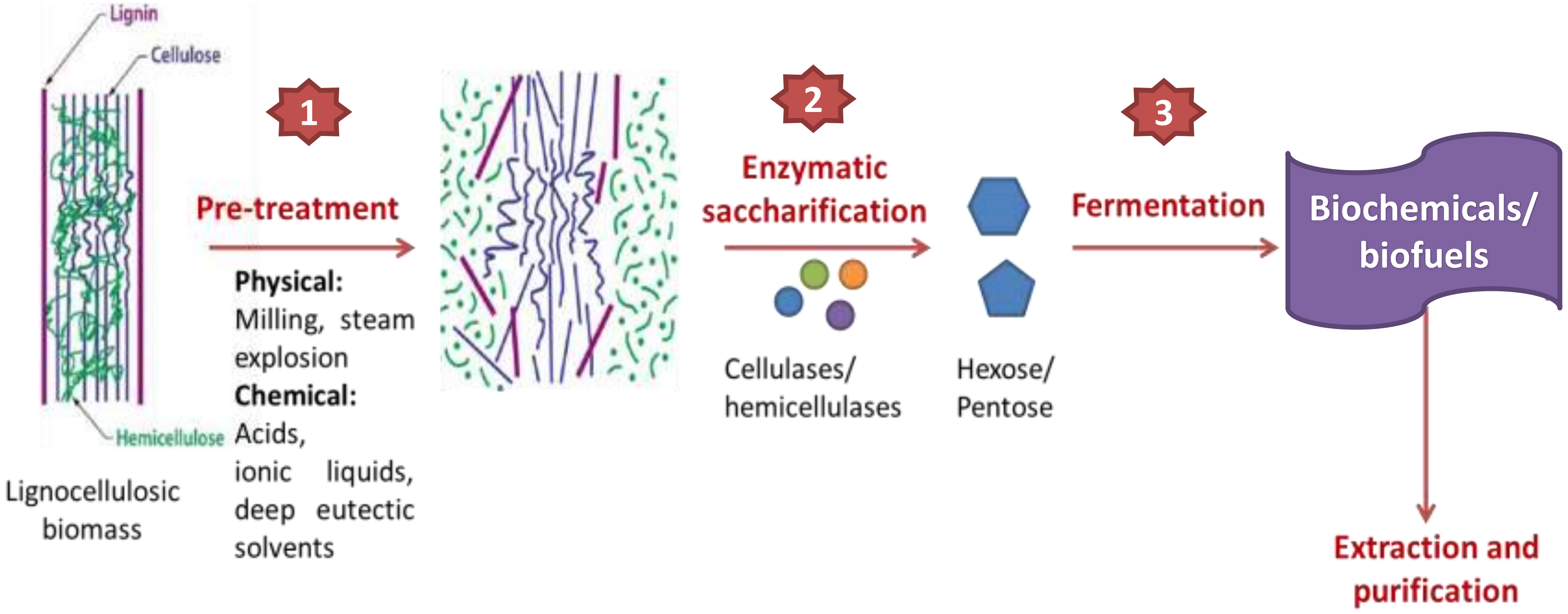
Average yields of organic acids produced from lignocellulosic biomass, compared to the theoretical maximum from glucose (100%)

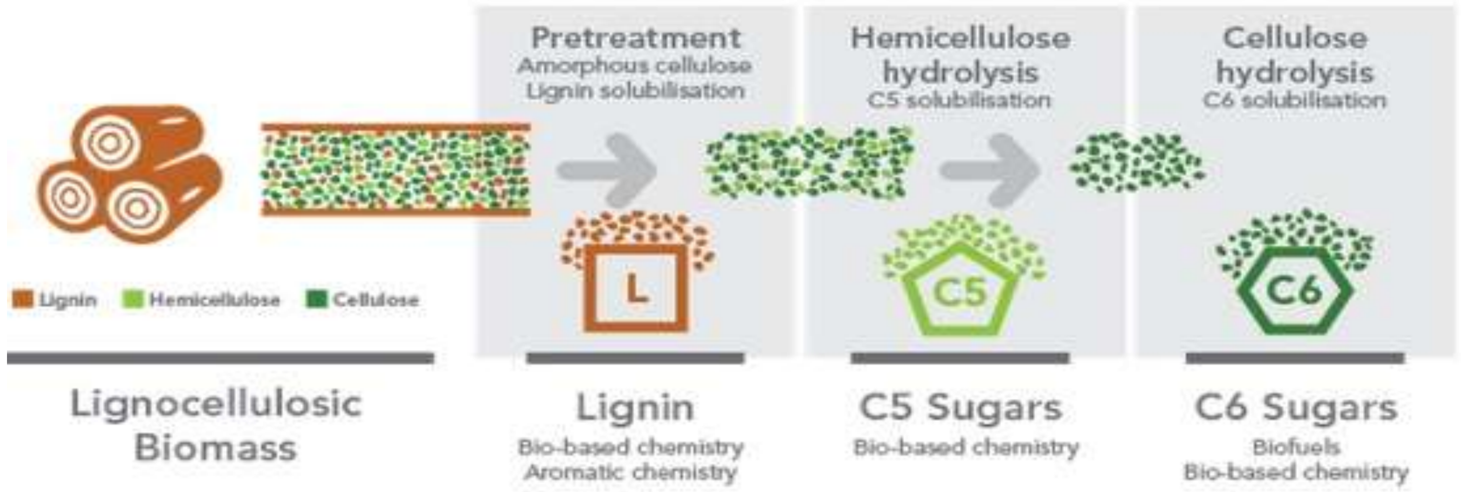


Júnior et al., 2021. *Biomass and Bioenergy*, *149*, 106092.

Steps for bioconversion of lignocellulosic biomass

1. Pre-treatment
2. Hydrolysis
3. Fermentation





Solid-state fermentation (SSF)

Biomass/ agro-industrial waste

↓

Adjustment of moisture level

↓

Sterilization

↓

Inoculation

↓

Incubation

↓

Extraction of fermentation product

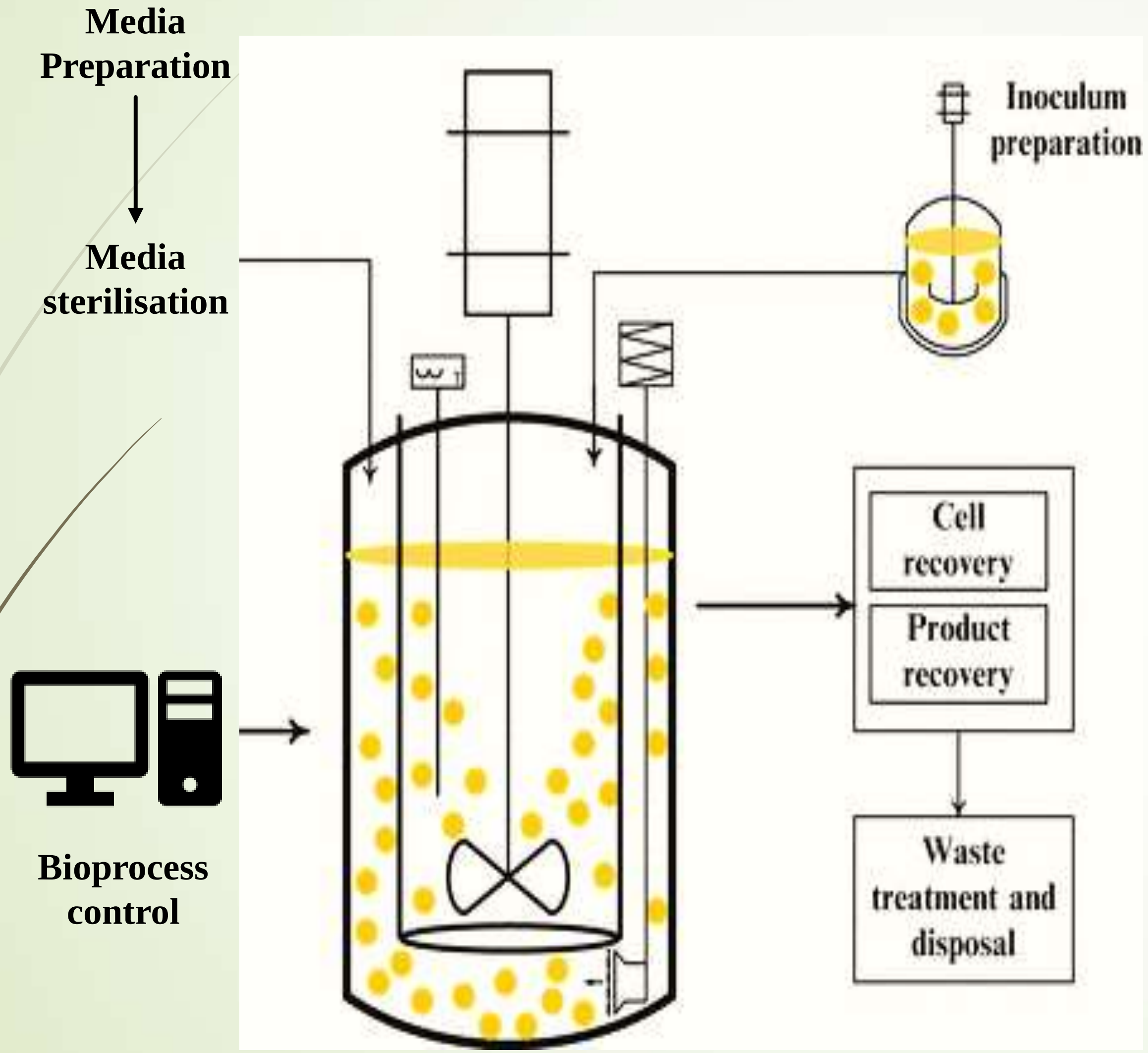
↓

Spent residue with improved nutritional quality can be used as feed or fertilizer

The advantages offered by SSF bioprocess:

- ✓ Economic feasibility as the agro-industrial residues/by-products are used as substrate in SSF, which bear very low or no cost.
- ✓ Production of concentrated end products in high titres and with robust stability.
- ✓ Lower energy demand and less risk of contamination.
- ✓ Mixed cultivation of microorganisms and fermentation of water-insoluble solid substrates.
- ✓ Valorisation and biological detoxification of large amounts of routinely generated agro-industrial residues/ by-products

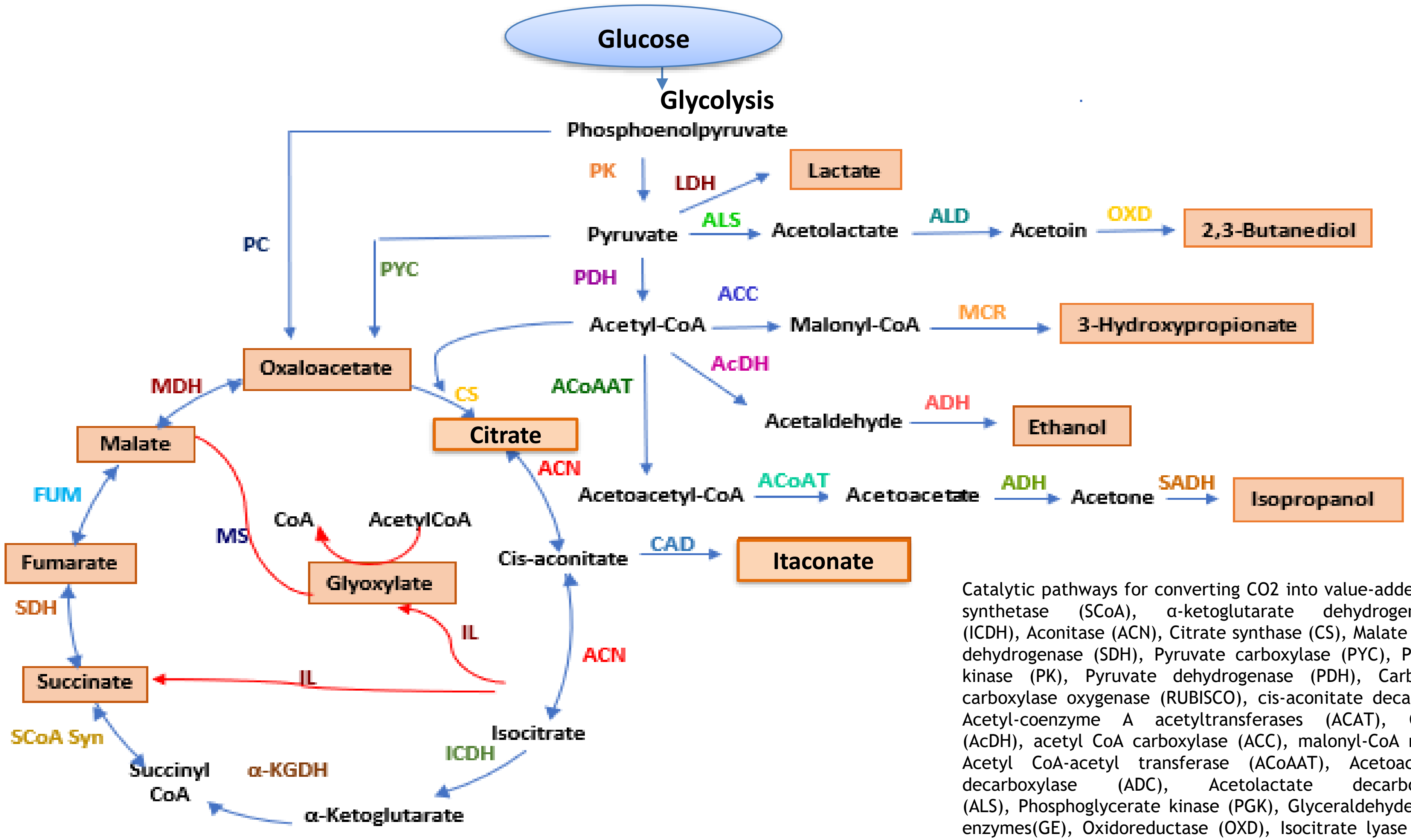
Submerged Fermentation (SmF)



The advantages offered by SmF bioprocess:

- ✓ Suitable for extraction of metabolites that need to be used in liquid form
- ✓ Traditionally used for the production of microbially derived enzymes.
- ✓ Short fermentation period with high product yields
- ✓ Purification of products is easier
- ✓ In liquid culture the control of the fermentation is simpler
- ✓ Decrease production costs by reducing the labor involved in solid-state methods.

Metabolic pathway for biosynthesis of commodity chemicals



Catalytic pathways for converting CO2 into value-added chemicals, materials, and fuels. (Succinyl CoA synthetase (SCoA), α-ketoglutarate dehydrogenase (α-KGDH), Isocitrate dehydrogenase (ICDH), Aconitase (ACN), Citrate synthase (CS), Malate dehydrogenase (MDH), Fumarase (FUM), Succinic dehydrogenase (SDH), Pyruvate carboxylase (PYC), Phosphoenolpyruvate carboxylase (PC), Pyruvate kinase (PK), Pyruvate dehydrogenase (PDH), Carbonic anhydrase (CA), Ribulose bisphosphate carboxylase oxygenase (RUBISCO), cis-aconitate decarboxylase (CAD), Lactate dehydrogenase (LDH), Acetyl-coenzyme A acetyltransferases (ACAT), CoA-acetylating acetaldehyde dehydrogenase (AcDH), acetyl CoA carboxylase (ACC), malonyl-CoA reductase (MCR), Alcohol dehydrogenase (ADH), Acetyl CoA-acetyl transferase (ACoAAT), Acetoacetyl-CoA transferase (ACoAT), Acetoacetate decarboxylase (ADC), Acetolactate decarboxylase (ALD), Acetolactate synthase (ALS), Phosphoglycerate kinase (PGK), Glyceraldehyde 3-phosphate dehydrogenase (GAPD), Glycolytic enzymes(GE), Oxidoreductase (OXD), Isocitrate lyase (IL), Secondary alcohol dehydrogenase (SADH), Malate synthase (MS)).

Zaidi et al., 2022. *Bioresource Technology,* 128174.

Our research towards biomass valorization into platform chemicals

Challenges biomass valorization into platform chemicals

- **Ligno-cellulosic complex- difficult to delineate and yields of products are often low**
- **Biomass to platform chemicals necessitates three steps process:**

  (i) Pre-treatment (ii) Saccharification (iii) Fermentation

  Such multistep processes make the process cost as unviable
- **The harsh pre-treatment methods are not eco-friendly and Industry averse**

  The by-products such as furfurals and hydroxyl methyl furfurals are inhibitory to enzymes and fermentative microorganisms in next steps
- **The saccharification requires cocktail of stable enzymes with high catalytic efficiency**
- **Efficient strains with appropriate metabolic flux for high product yields**
- **Coupled down stream**

Our strategies

- Solid State Fermentation

  - Biomass is directly used as substrate for production of platform chemicals

- Unutilizable biomass due to toxic substances

  - Use as substrate for production of platform chemicals with simultaneous detoxification

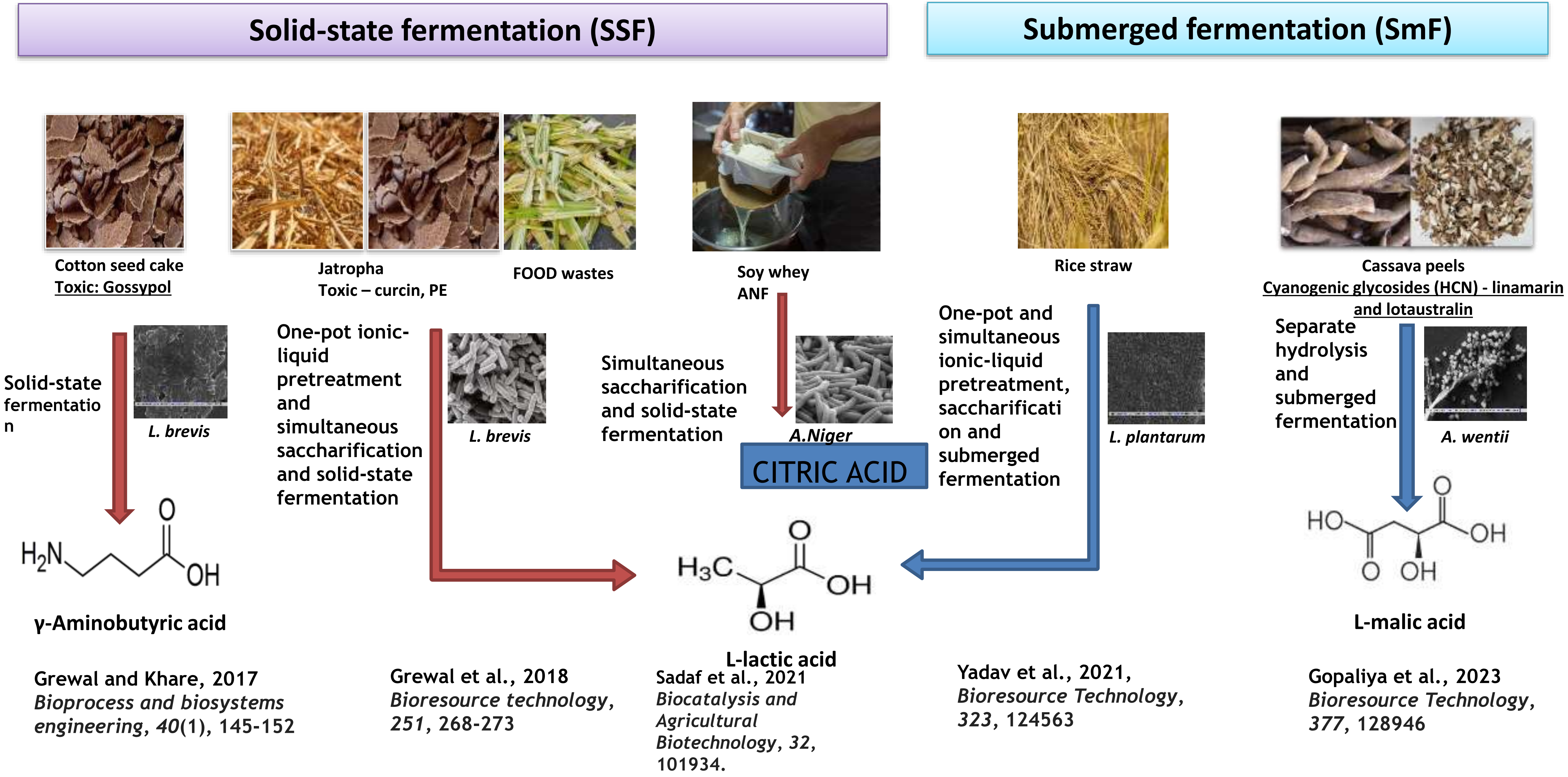
- Use of ionic liquids/ eutectic solvents

  - Environment friendly green approach for pre-treatment

- Use of ionic liquids/ eutectic solvents stable enzymes and microbial cells

  - One pot process for pre-treatment, saccharification and fermentations

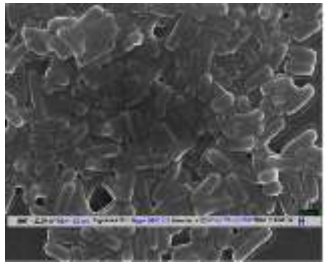
Biomass valorization into platform chemicals



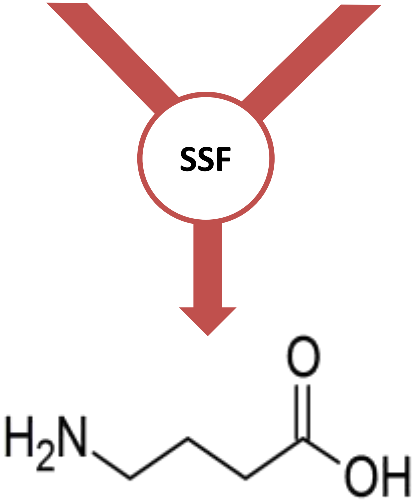
Solid state fermentation of toxic cotton seed cake: γ-Aminobutyric acid

- **γ-Aminobutyric acid** (GABA), is a C4-platform chemical as well as a non-protein amino acid which find applications in pharmaceutical, nutraceutical and polymer industry
- **Cottonseed cake (having toxic gossypols)** was used a feedstock for GABA production through **Solid state fermentation** using *Lactobacillus brevis*

Cotton seed cake



L. brevis



γ-Aminobutyric acid

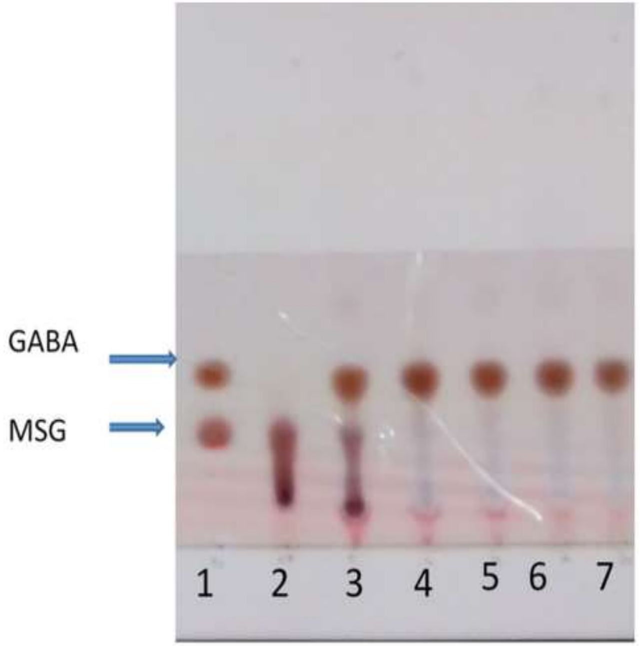


Fig. 2 Thin-layer chromatography of cottonseed cake extract after SSF with *L. brevis*. *Lanes 1* standard GABA and MSG; *2* abiotic control (extract from unfermented cottonseed cake); *3–7* extract of *L. brevis* fermented cottonseed cake after 2, 3, 6, 9, and 12 days, respectively

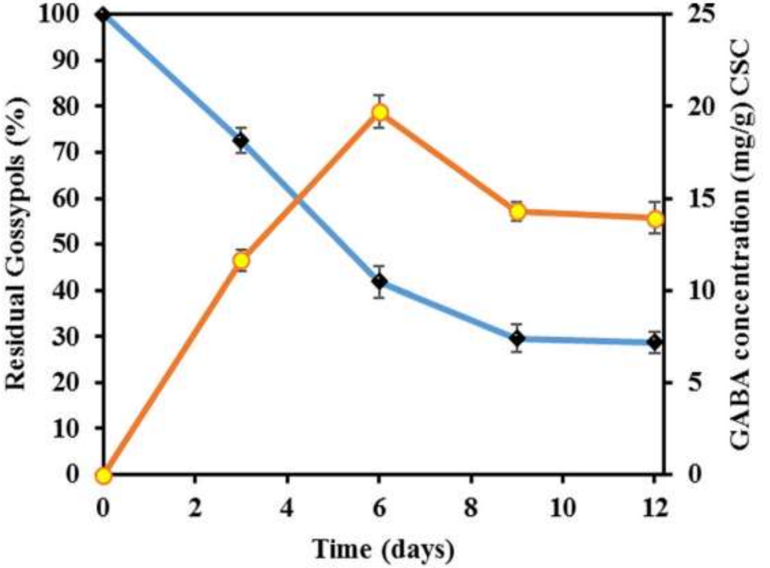
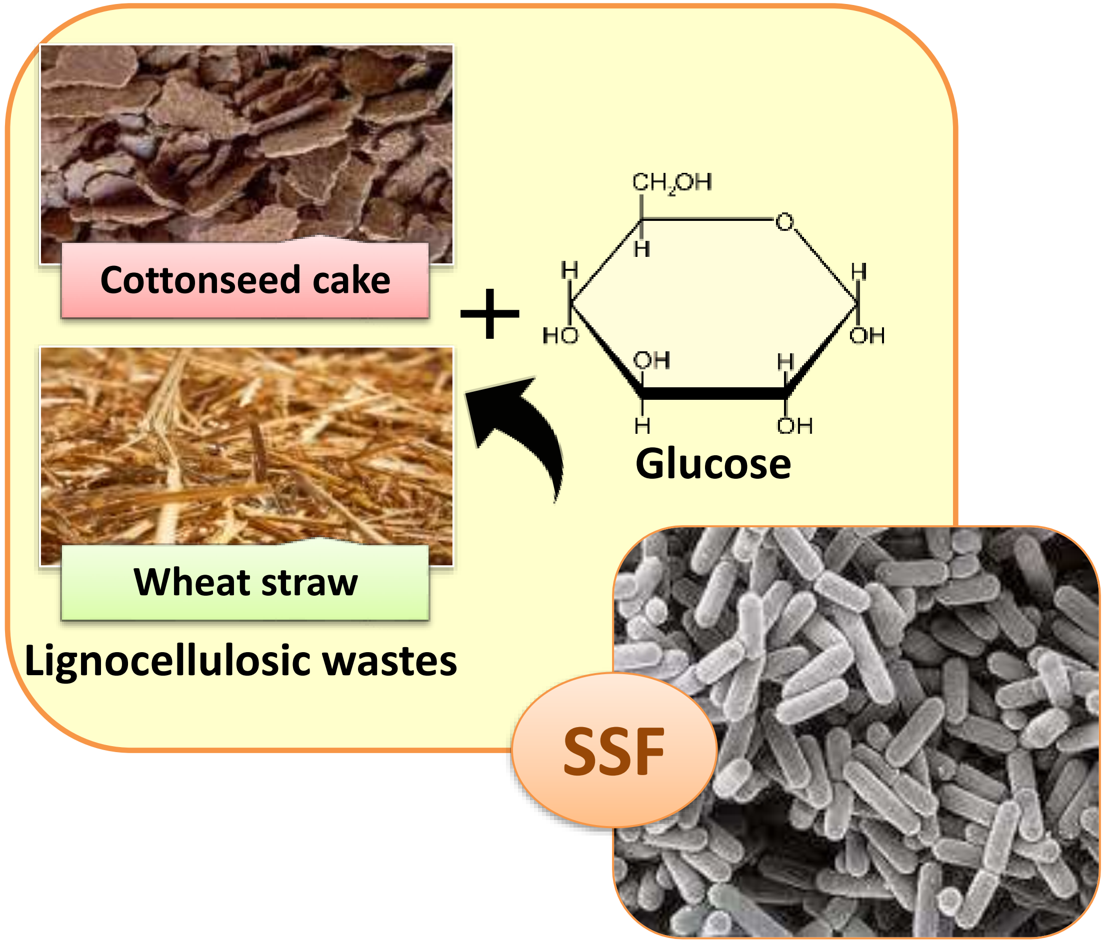


Fig. 3 GABA production with simultaneous degradation of gossypols in cottonseed cake fermentation by *L. brevis* under optimized conditions. Five grams cottonseed cake moistened to 1:3 (w/v) by sodium phosphate buffer (0.1 M, pH 6.0), supplemented with 5 % (w/w) MSG and 200 μM (PLP) was seeded with 2-ml inoculum (9.89 log cfu/ml) of *L. brevis* and incubated at 30 °C. *Filled circles* represent the GABA concentration (mg/g CSC) and *filled rhombus* represent the residual gossypols (%)

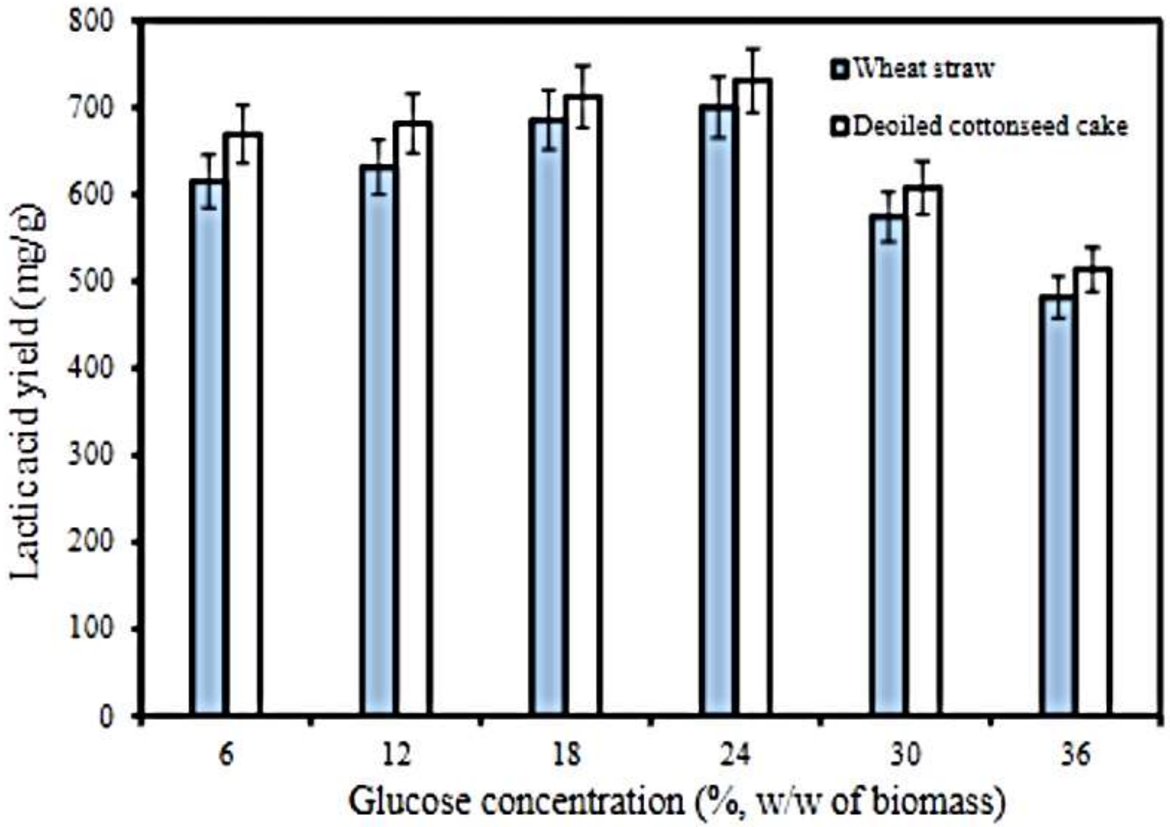
Solid state fermentation: Lactic acid

The optimized **SSF** conditions of lignocellulosic wastes supplemented with 24%(w/w) glucose resulted in lactic acid production

- **Cottonseed cake : 740mg/g**
- **Wheat straw : 700 mg/g**



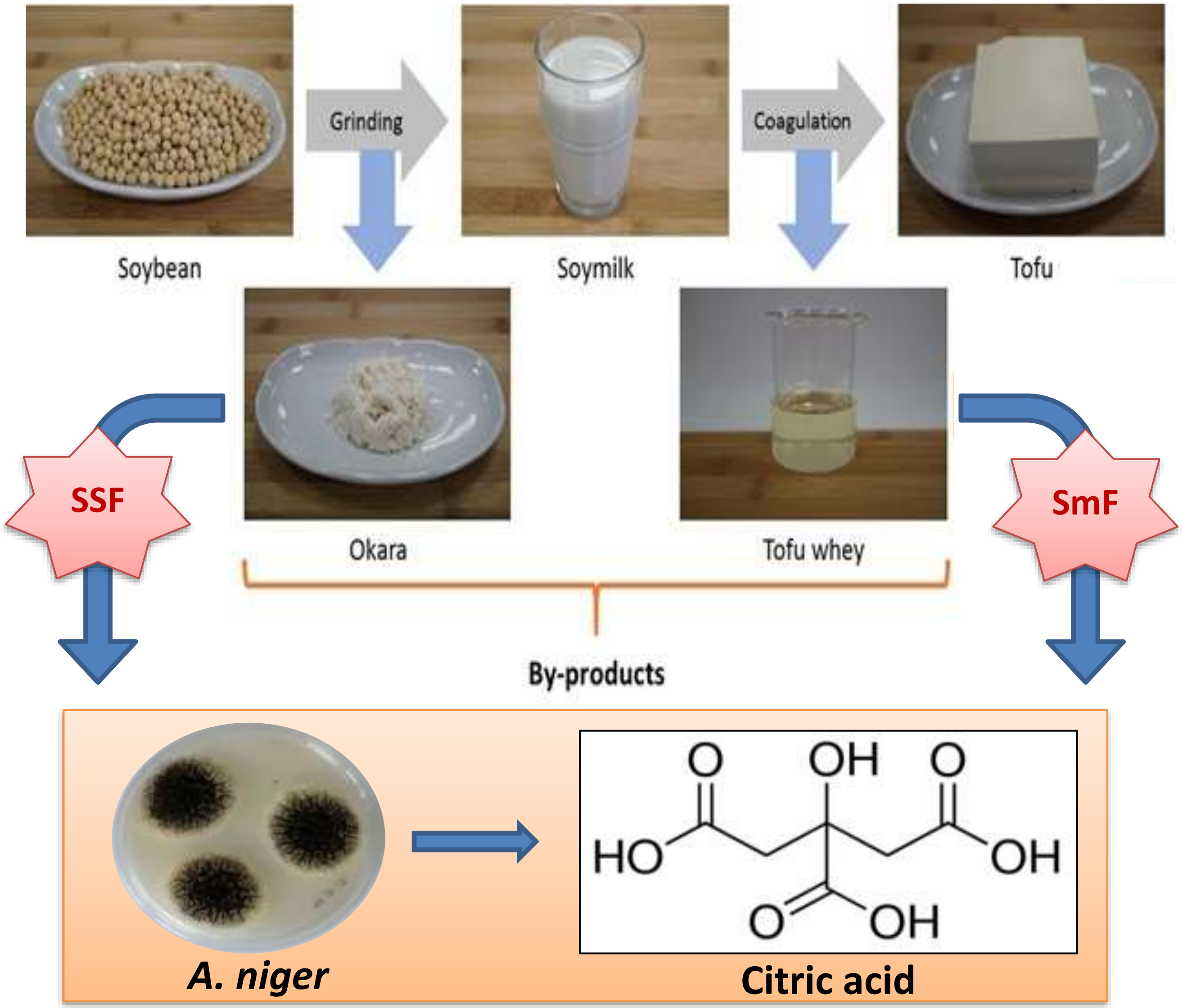
L. brevis



Lactic acid yield in solid state fermentation (SSF) using wheat straw and cottonseed cake as solid nutrient supports supplemented with different concentrations of glucose (6-36%w/w)

Citric acid from Okara (SSF) and Soywhey (SmF)

Soy industry by-products were used as feedstock for fermentative production of citric acid using *Aspergillus niger*



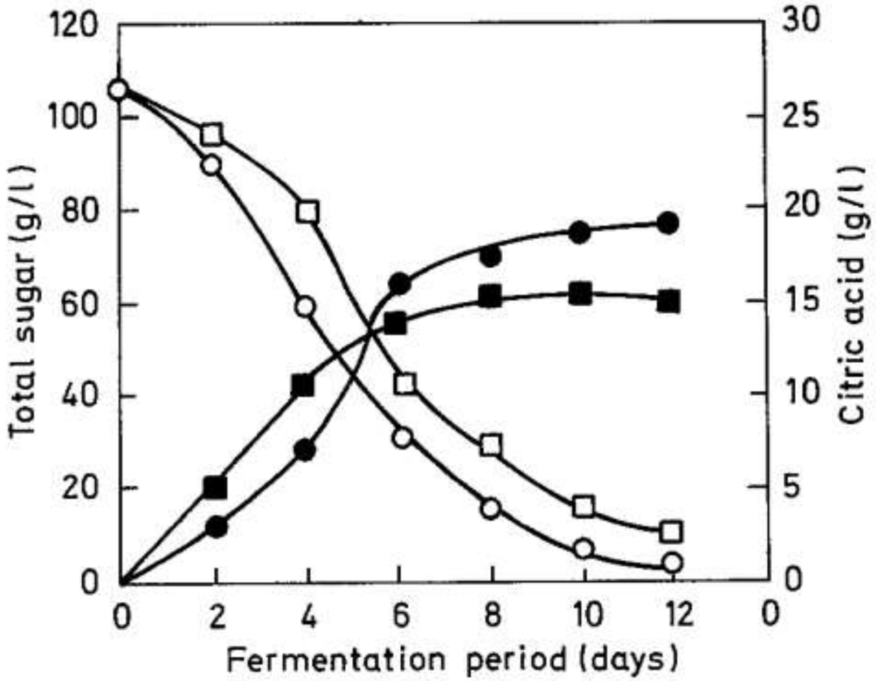


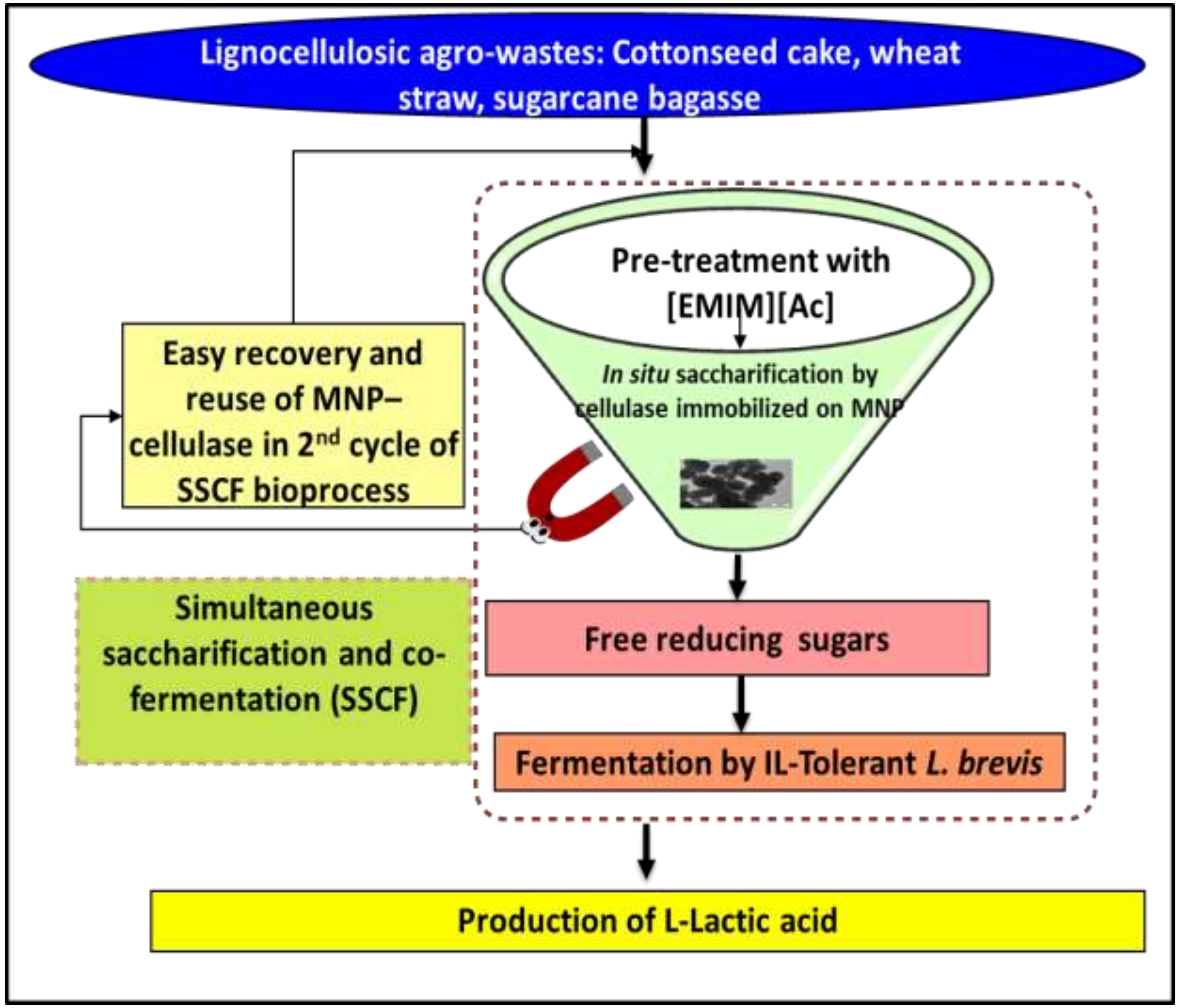
Fig. 1. Citric acid production and sugar consumption by free and immobilized *Aspergillus niger* cells with respect to time. Soy whey (100 ml) as inoculated with 10% cells (w/v), incubated at 30°C and constantly shaken at 200–220 rpm. Citric acid was estimated at various time intervals: □, ■, free cells; ○, ●, immobilized cells; ■, ●, citric acid; □, ○, sugar

Agro wastes- Lactic acid

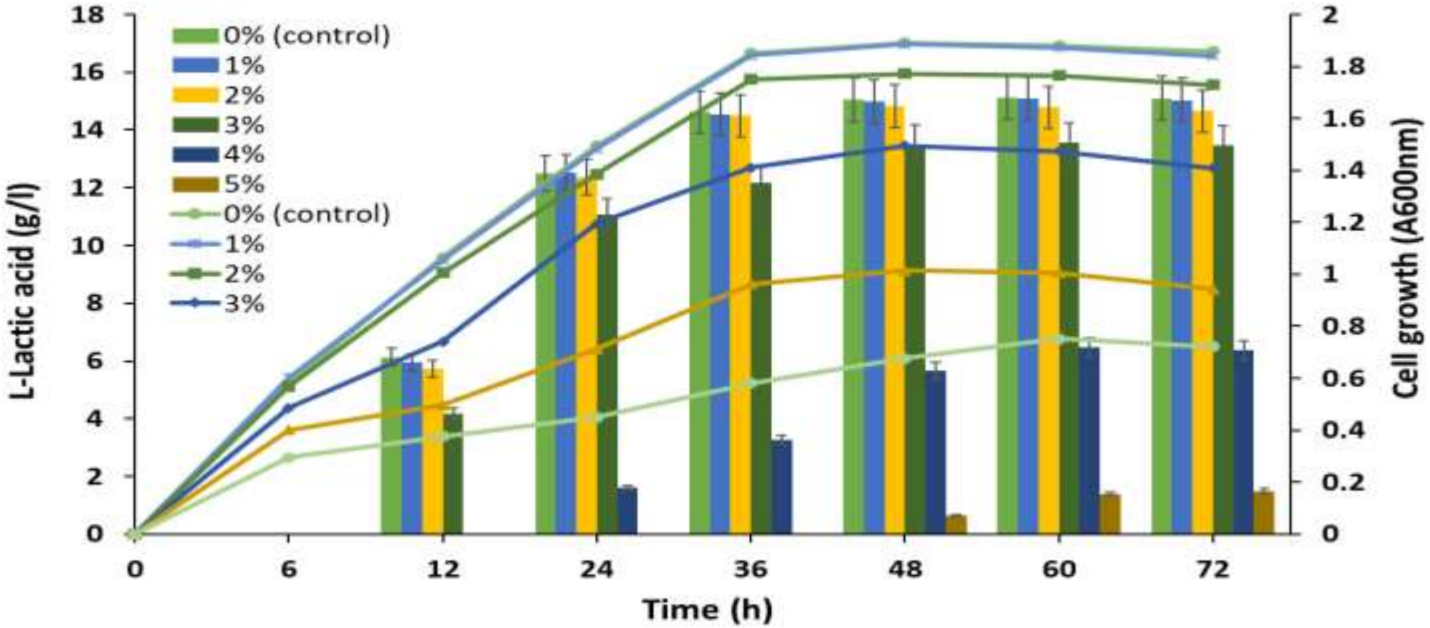
One-pot Bio process: Pre- treatment, saccharification and solid state fermentation

- A generic **one-pot bioprocess** was developed for lactic acid production from lignocellulosic agro-waste
- **Simultaneous saccharification and co-fermentation (SSCF)** was conducted in a **solid-state bioprocess**
- Ionic-liquid tolerant *L. brevis* was used
- Nano-immobilized ionic-liquid stable cellulase used for saccharification was easily recoverable by a magnet and reused for next cycle of SSCF
- The SSCF process generated lactic acid

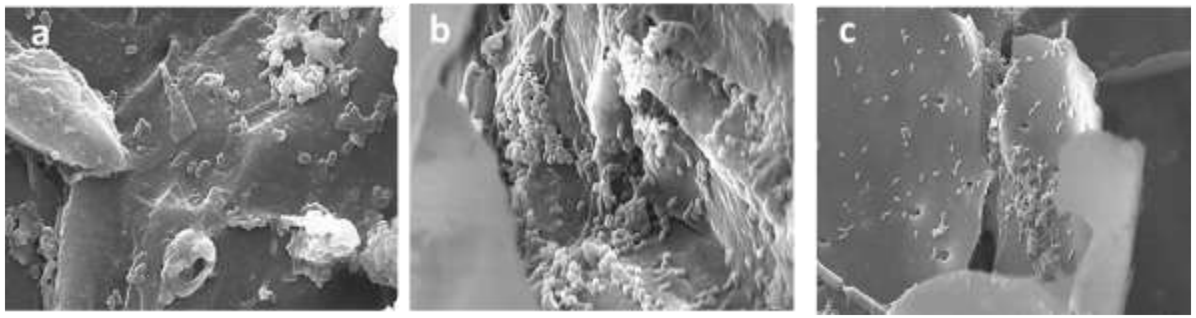
- **Cottonseed cake – 220 mg/g**
- **Wheat straw – 490 mg/g**
- **Sugarcane bagasse – 520 mg/g**



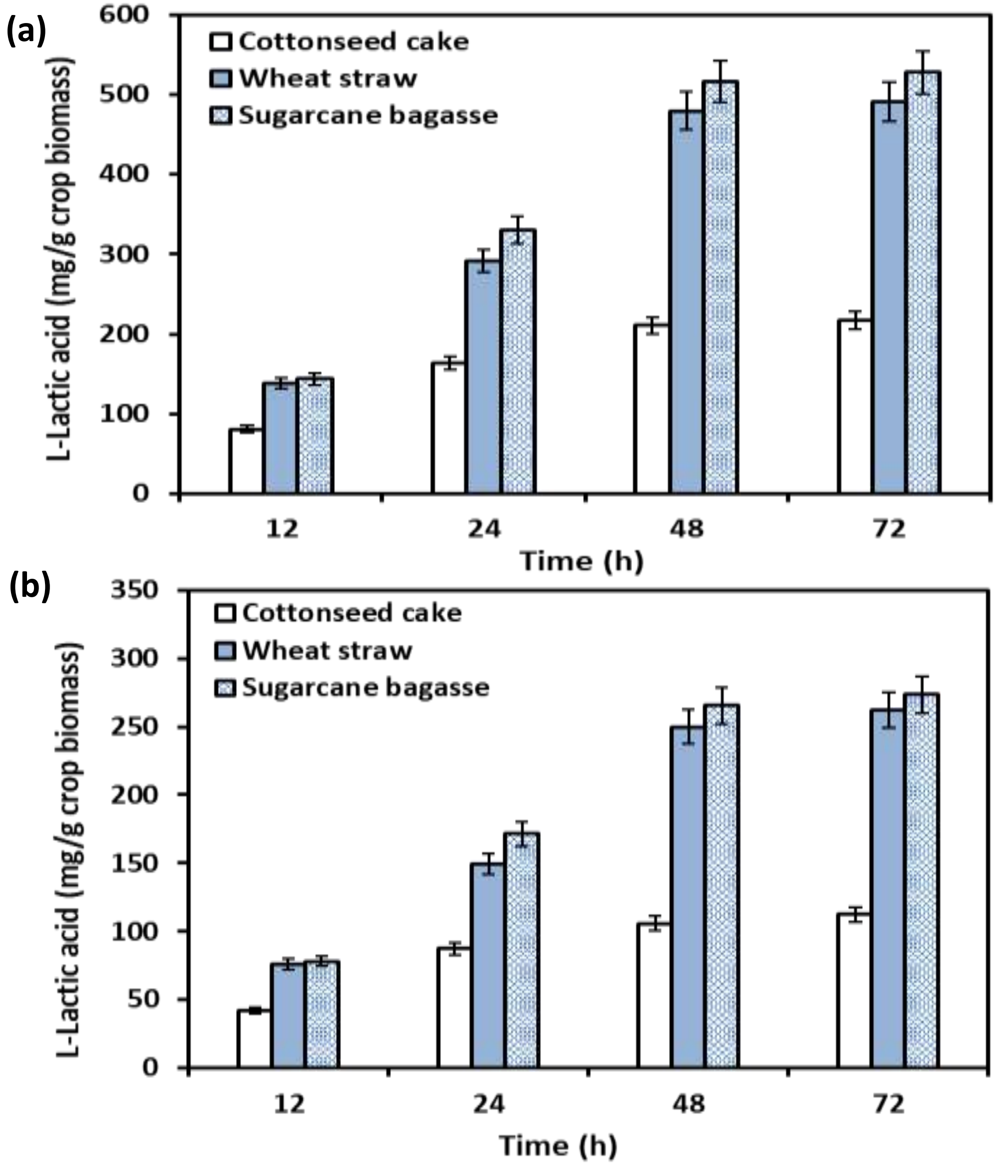
One-pot bioprocess for lactic acid production from solid agro-wastes



L. brevis cells could grow in the presence of [EMIM][Ac] and produce lactic acid without any inhibition till 3%(v/v) [EMIM][Ac]



Scanning electron micrographs of *L. brevis* adhered to [EMIM][Ac] pretreated feedstocks during one-pot SSCF process (a) fermented cottonseed cake (b) fermented wheat straw (c) fermented sugar cane bagasse

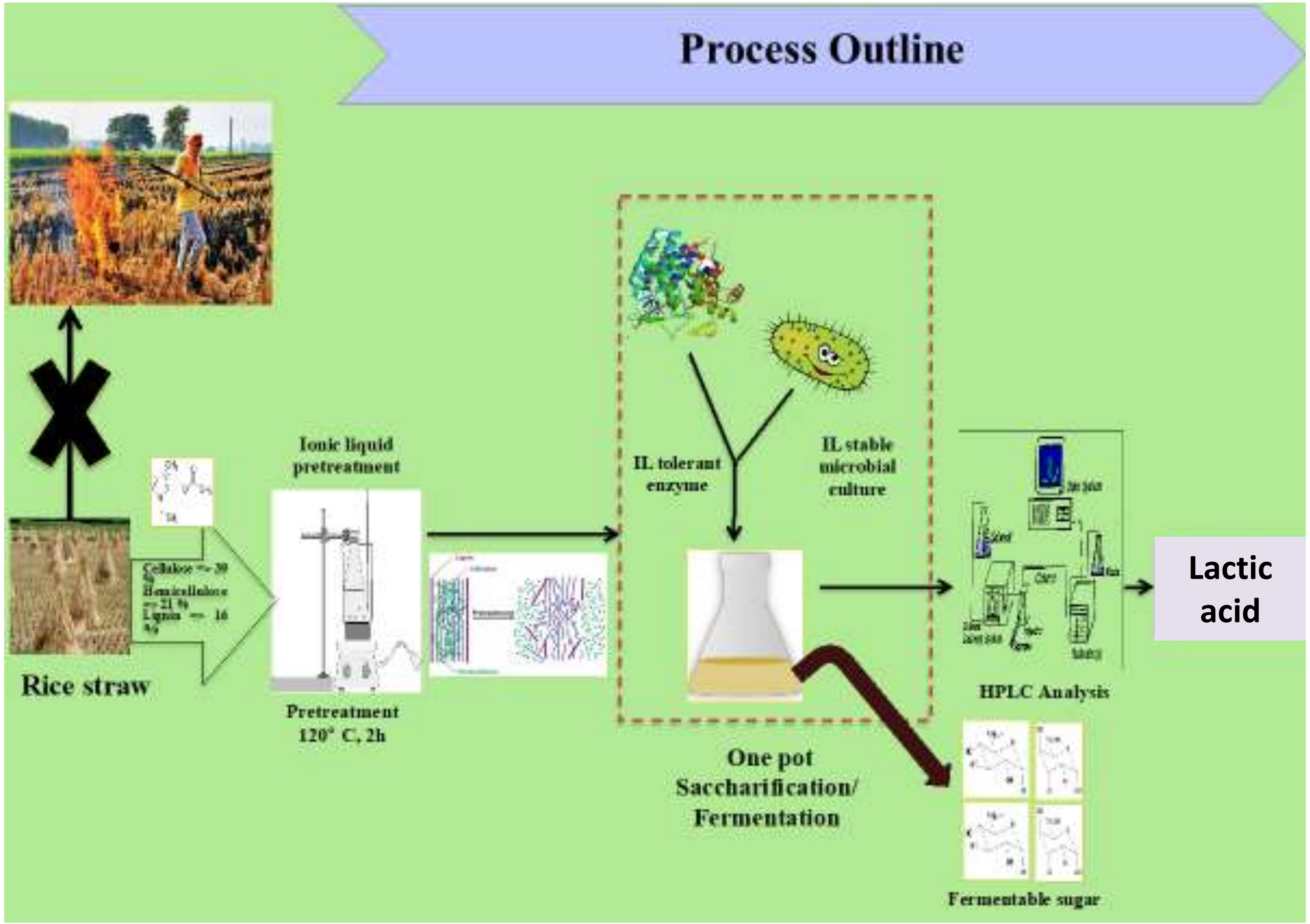


Lactic acid production from [EMIM][Ac] pretreated agro-waste feedstocks, hydrolysed by MNP-immobilized cellulase and fermented by *L. brevis* (a) SSCF process usingMNP-immobilized cellulase (b) SSCF with reused MNP-immobilized cellulase

Rice straw -Lactic acid

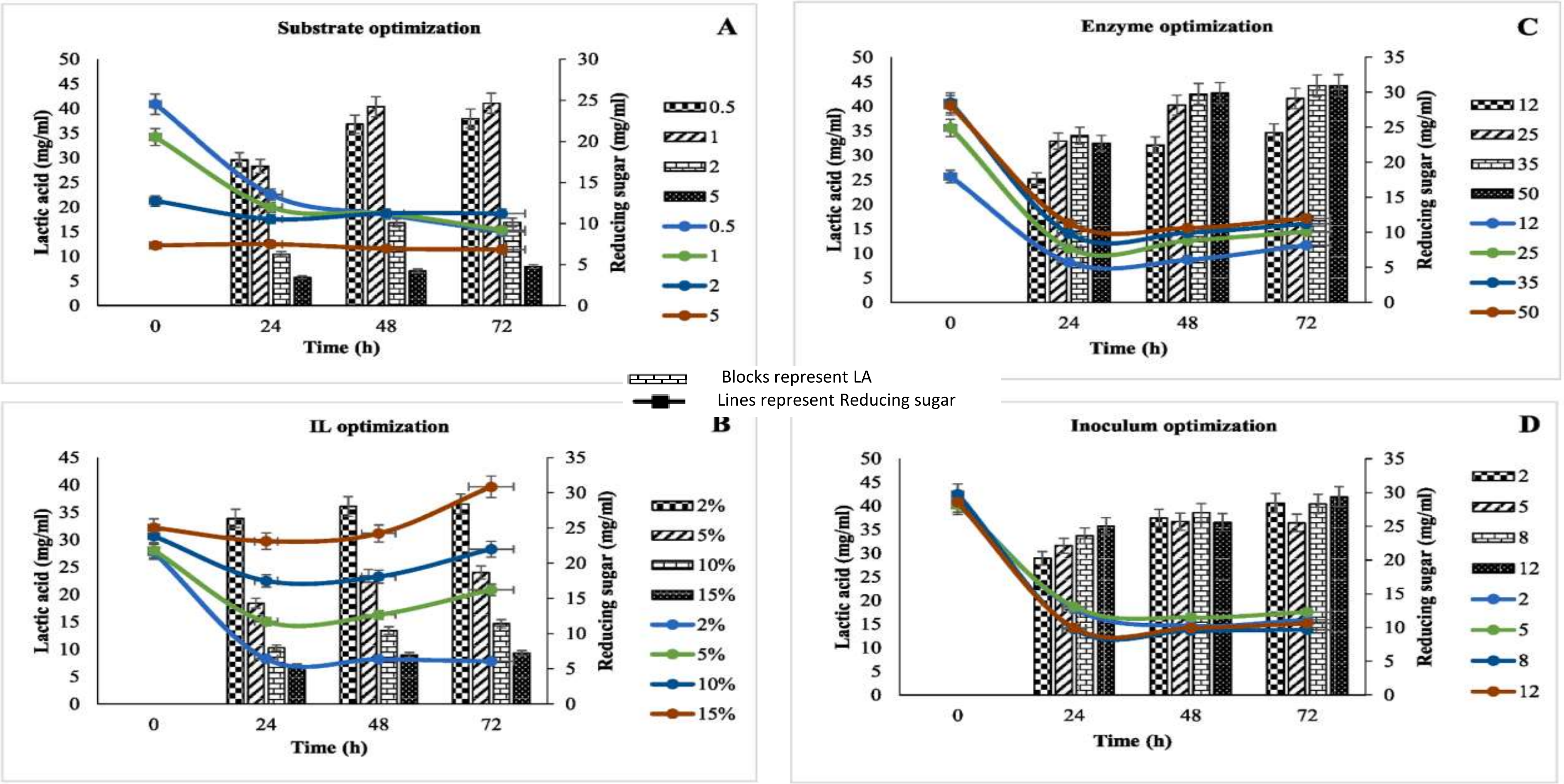
One-pot Bio process: Pre- treatment, saccharification and submerged fermentation

- An Ionic liquid stable *Lactobacillus plantarum* isolate *was used* for lactic acid production
- **One-pot pretreatment (ionic liquid - [EMIM][OAc]) and enzymatic hydrolysis (Cellic Cellic CTec2)** making the process economical and feasible.
- The process using **rice straw** as substrate led to a **LA yield of 36.75 g/L** from *L. plantarum* SKL-22 in a single pot bioprocess.
- In a 5 L bioreactor, the process was further upscale, and **a yield increment of 1.11 %** was observed.



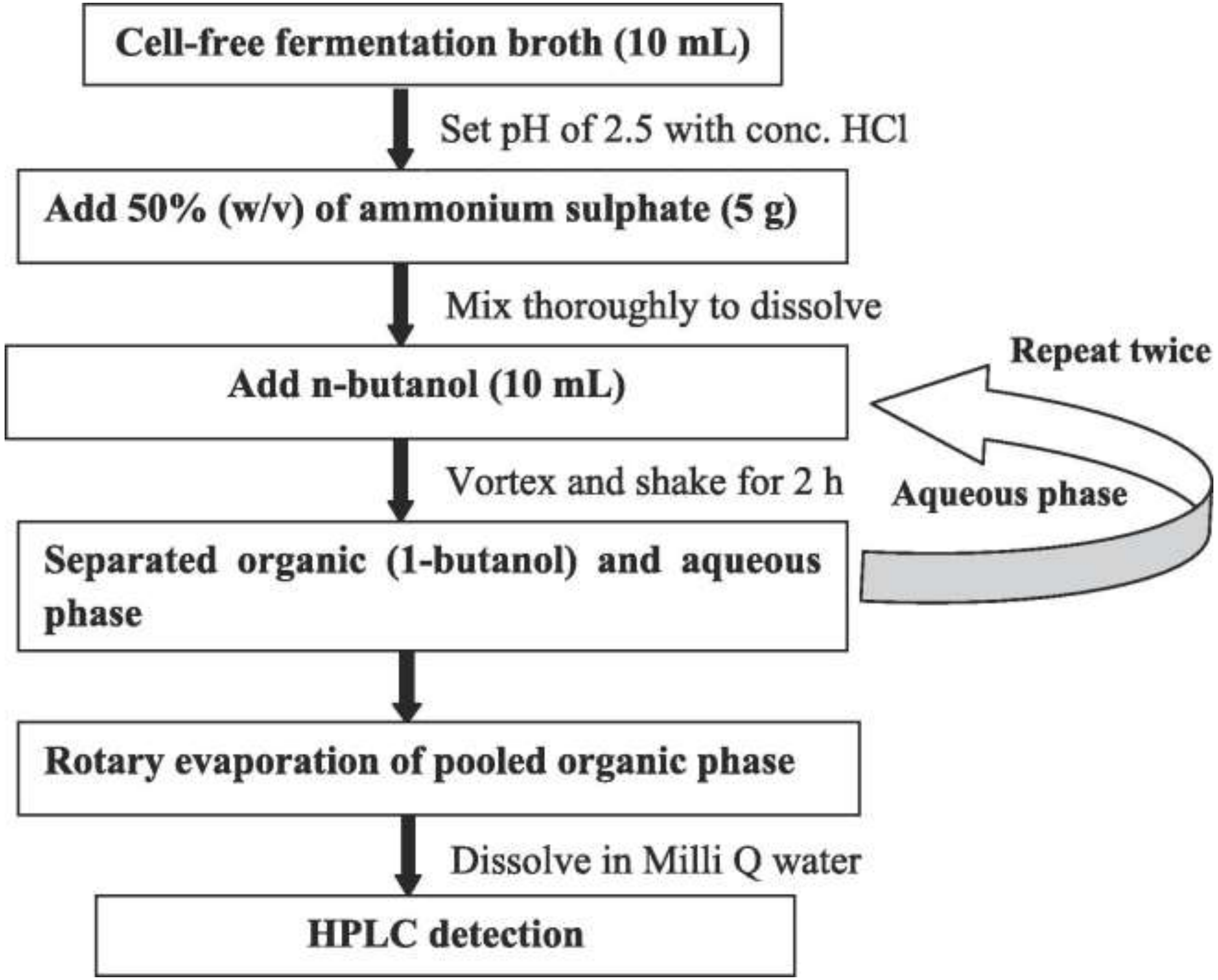
One-pot bioprocess for lactic acid production from wheat straw

Optimization of various parameters for the production of lactic acid



Parameter	Substrate (rice straw)	Ionic liquid [EMIM][OAc]	Enzyme (Cellic CTec2)	Inoculum	Yield
Optimized condition	1 g	2% (v/v)	25 FPU/g	12%	36.75 g/L

A simple downstream processing protocol for the recovery of lactic acid from the fermentation broth



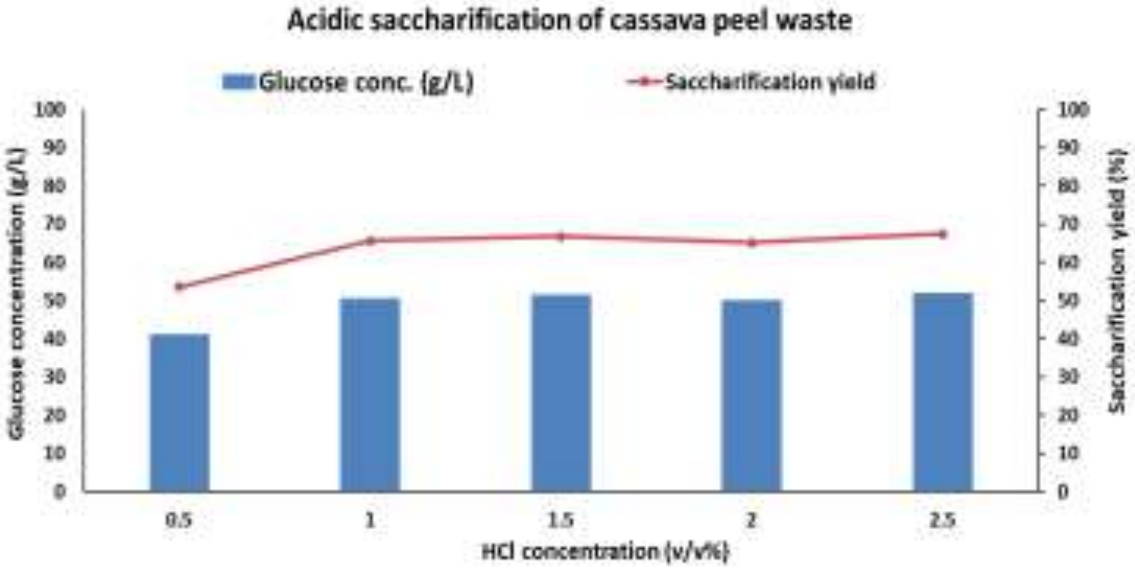
- Phase partitioning using n-butanol has been developed as a simple and economical method for downstream lactic acid processing.
- The pH of the extraction medium was critical during LA purification.
- Under the LA downstream process's optimized conditions, a yield of 86% and 93% purity was obtained.

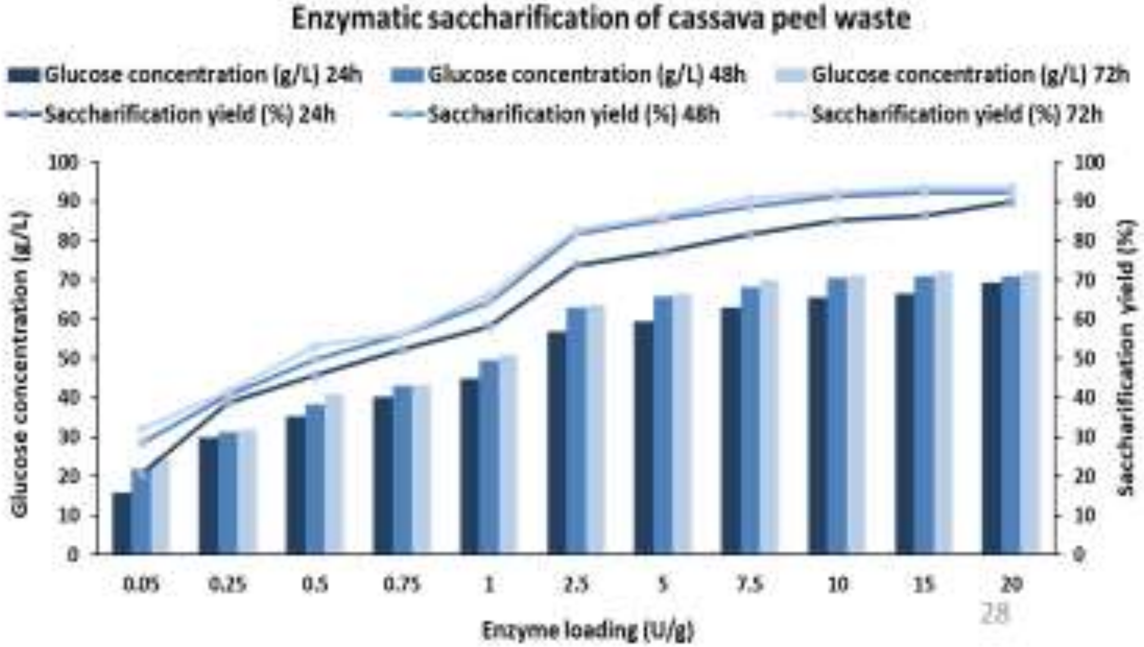
Toxic cassava peel waste: Malic acid

- **Malic acid** is an important platform chemical with applications in food & beverage, polymer, pharmaceutical, and textile industries.
- **Cassava (Tapioca) peel waste** was used as a low-cost feedstock for production of malic acid using *Aspergillus wentii*
- Cassava peel was saccharified through acidic and enzymatic treatment prior to fermentation

Acidic saccharification: HCl
Acid loading: 1%(v/v) **Solid loading:** 20%(w/v)

Enzymatic saccharification: Amyloglucosidase
Enzyme loading: 2.5U/g **Solid loading:** 15%(w/v) **Treatment time:** 48h

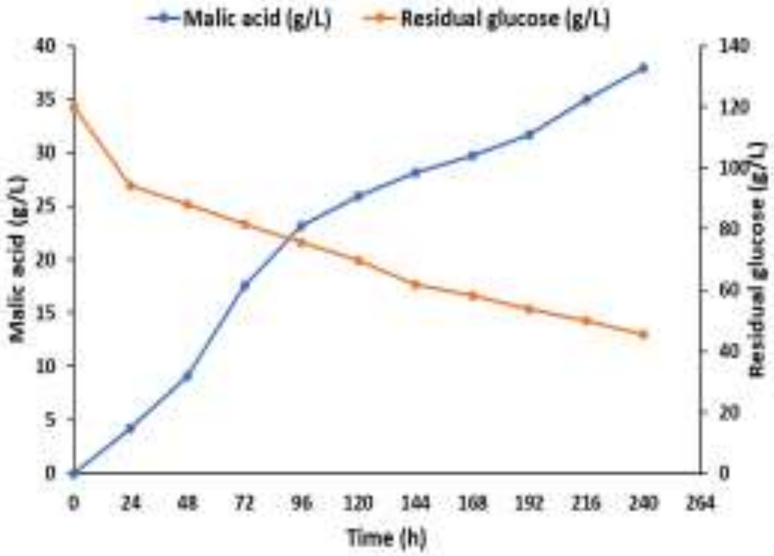




Fermentation of cassava peel hydrolysate for malic acid production

Glucose rich hydrolysates (acidic/enzymatic) were used as low-cost media for **submerged fermentation (SmF)**

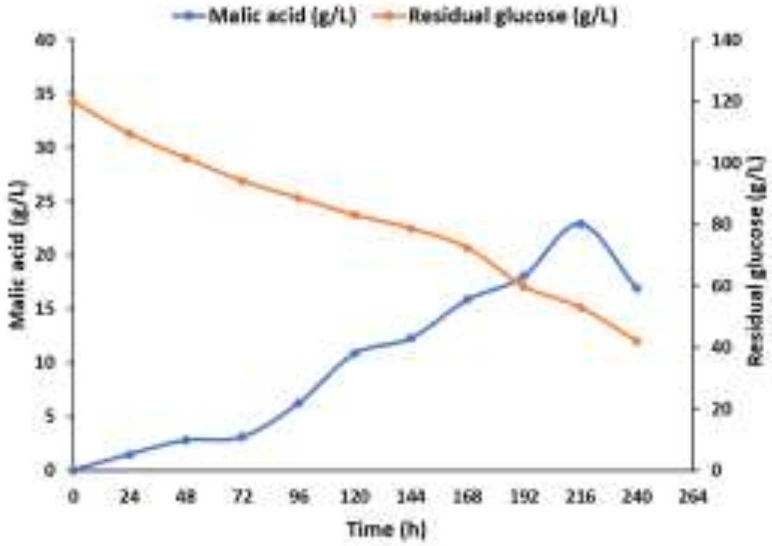
Time profile of malic acid production pure glucose



***Maximum values attained at 240h (10 days of fermentation)**

Titer	:	**37.9 g/L**
Yield	:	**0.5 g/g**
Productivity	:	**0.157 g/L/h**

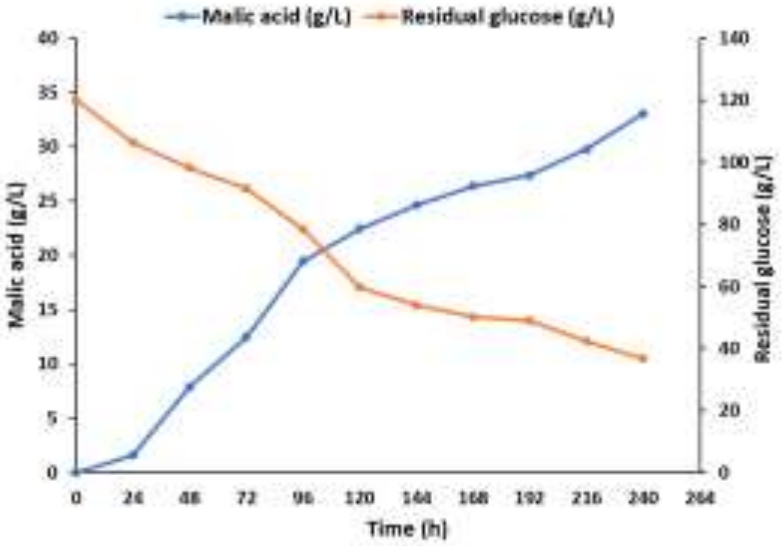
Time profile of malic acid production from acidic hydrolysate of cassava peels



***Maximum values attained at 216h (9 days of fermentation)**

Titer	:	**22.94 g/L**
Yield	:	**0.34 g/g**
Productivity	:	**0.106 g/L/h**

Time profile of malic acid production from enzymatic hydrolysate of cassava peels



***Maximum values attained at 240h (10 days of fermentation)**

Titer	:	**33.06 g/L**
Yield	:	**0.39 g/g**
Productivity	:	**0.137 g/L/h**

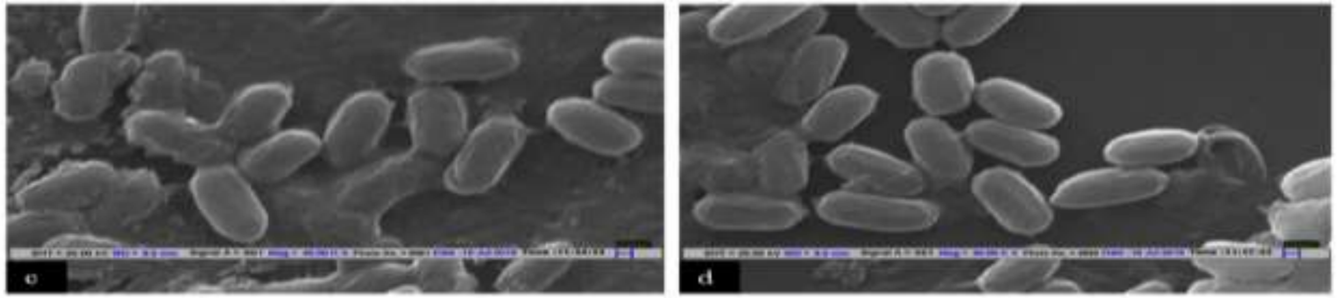
Single-cell oil production from starchy wastes

Valorization of agro-starchy wastes as substrates for oleaginous yeasts

Lipid productivity (g/g starch)

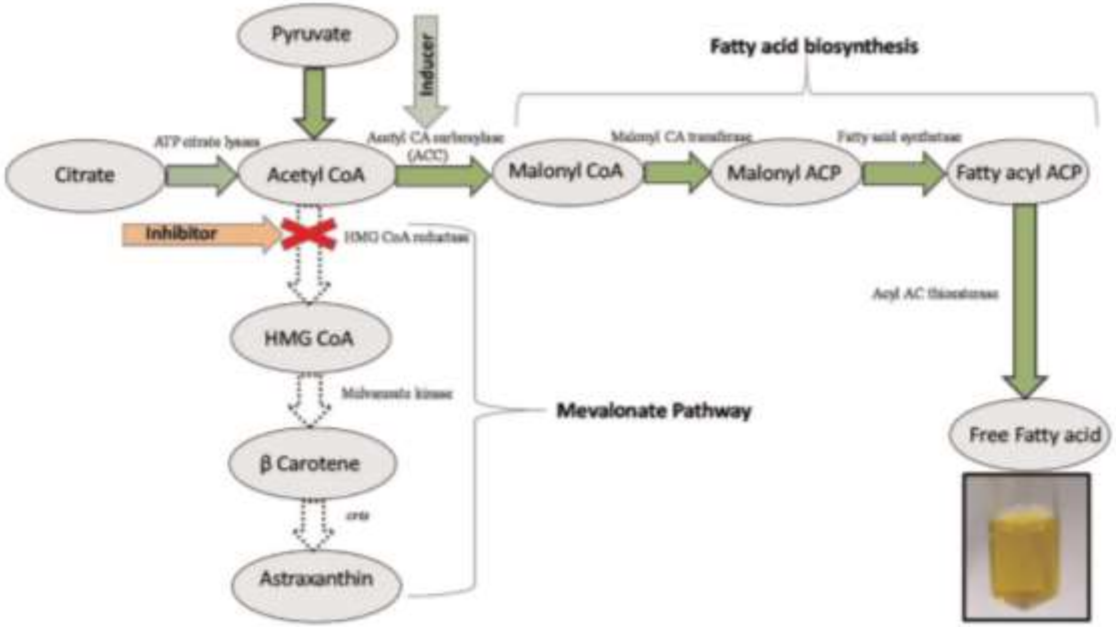
Substrate	*R. glutinis*	*R. mucilaginosa*	*S. pastorianus*
Potato peel	5.1 ± 0.10	6.0 ± 1.00	4.7 ± 1.10
Banana peel	5.8 ± 1.60	6.0 ± 1.53	5.7 ± 3.10
Cassava peel	4.5 ± 0.70	3.6 ± 0.60	5.2 ± 0.20
Corn residue	6.7 ± 0.70	5.9 ± 0.67	4.7 ± 1.10
Rice residue	5.6 ± 1.80	5.5 ± 0.57	4.1 ± 1.10
Wheat bran	4.6 ± 0.00	3.9 ± 0.76	3.3 ± 0.50
Yam peel	5.6 ± 2.20	3.0 ± 0.42	3.9 ± 2.10
Barley residue	5.3 ± 0.90	8.3 ± 5.70	2.9 ± 0.50

Substrate	*T. cutaneum*	*L. starkey*	*C. curvatus*
Potato peel	5.2 ± 0.40	4.7 ± 1.70	4.1 ± 0.70
Banana peel	5.7 ± 2.50	14.3 ± 10.30	2.2 ± 0.00
Cassava peel	8.1 ± 1.30	8.0 ± 2.20	3.1 ± 0.70
Corn residue	4.3 ± 1.10	5.3 ± 0.50	3.3 ± 0.30
Rice residue	13.9 ± 2.30	6.1 ± 2.90	6.2 ± 1.20
Wheat bran	2.0 ± 0.60	16.0 ± 11.0	2.7 ± 0.30
Yam peel	4.1 ± 0.70	5.6 ± 1.80	2.5 ± 0.10
Barley residue	4.9 ± 0.70	6.8 ± 0.80	4.5 ± 1.50
	2.60	8.73	1.45



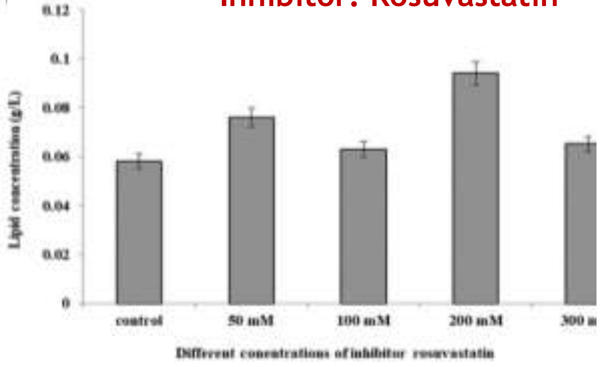
SEM image showing lipid production by *R. glutinis* on cassava residues (SmF)

Inducing and repression of key rate-limiting enzymes (acetyl CoA carboxylase and HMG reductase) to enhance fatty acid production from *Rhodotorula mucilaginosa*

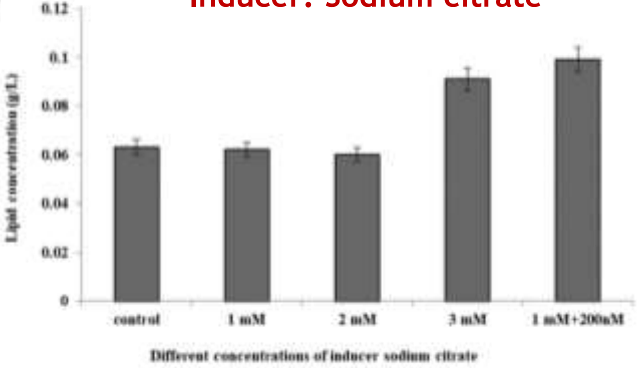


Effect on lipid production

Inhibitor: Rosuvastatin



Inducer: Sodium citrate



- Our research on biomass valorization for production of platform chemicals indicate:

1. **Un-utilizable/ toxic agro wastes could be good substrates**

(simultaneous detoxification thus addressing their disposal problems)

2. Solid state fermentation can be done directly

(making process simpler)

3. **Pre-treatment with ionic liquids / eutectic solvents**

(Environmental friendly, inhibitors for saccharification and fermentation are not there, process could be one pot)

4. **Use immobilized saccharification hydrolases**

(become stable and can be recovered and reused)

5. **Efficient fermentative strains**

(stable in solvents and ferment in one pot)

6. **Coupled down stream**

(makes process viable)

Thus SSF of agrowastes with one pot process (by employing green solvents and immobilized enzyme and stable organisms) coupled with down stream is an attractive approach in Biorefinary for Biomass valorization towards cost-effective production of commodity chemicals

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Thank you