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AGRICULTURAL UNIVERSITY OF ATHENS

# Optimization of fermentation parameters for enhanced poly(3-hydroxybutyrate) production

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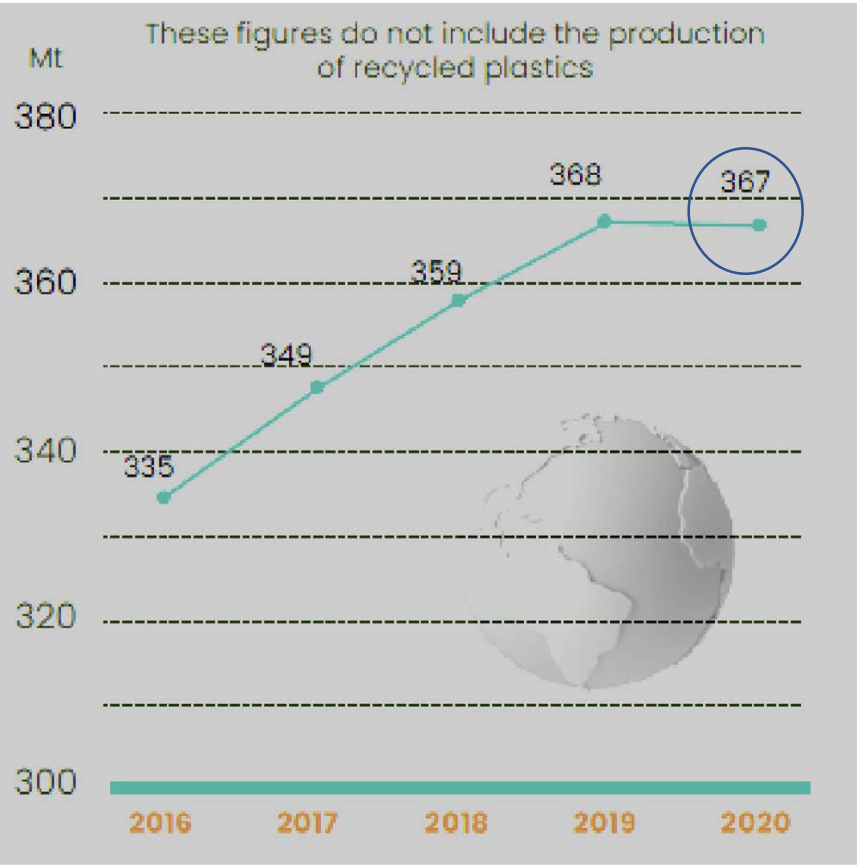
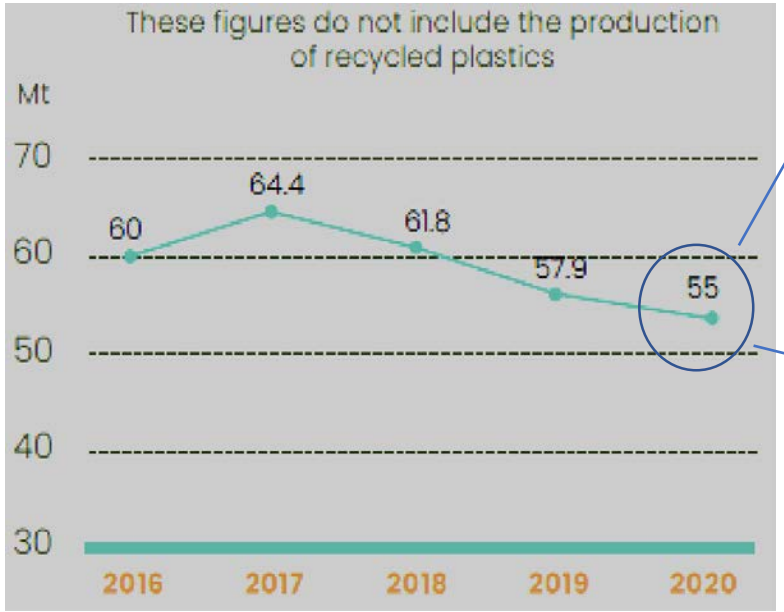
## WORLD PLASTICS PRODUCTION*




## EUROPEAN PLASTICS PRODUCTION*




29.5 Mt
Collected plastic

ENERGY RECOVERY 42%

RECYCLING** 34.6%

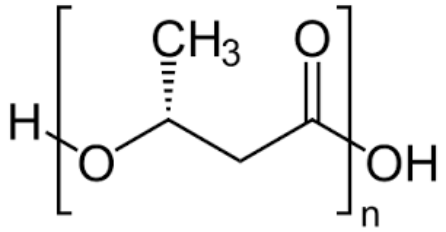
LANDFILL 23.4%

25.5 Mt unmanaged plastics

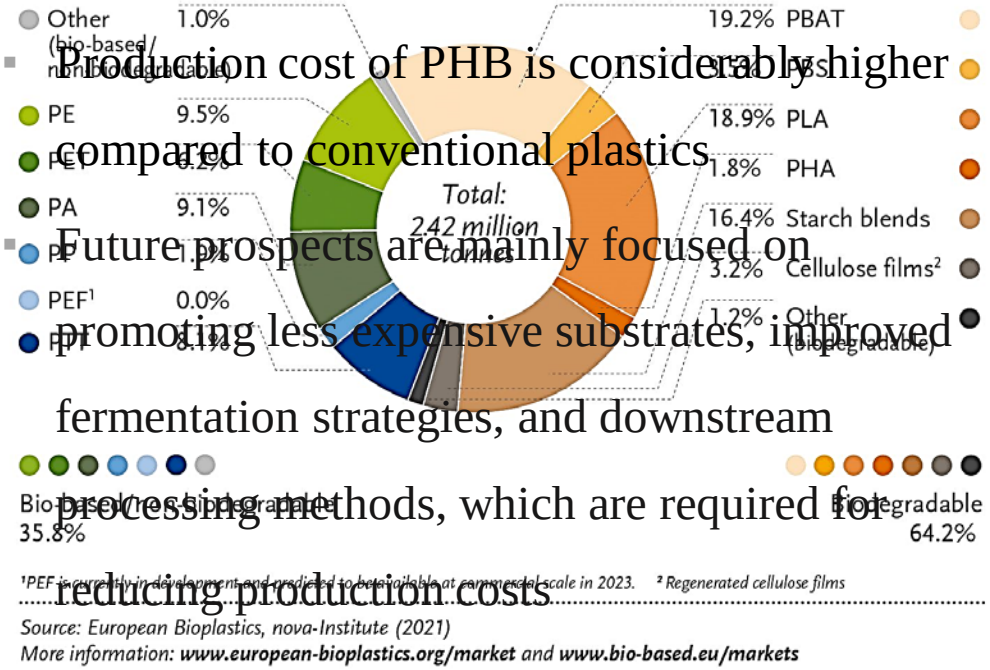
*Plastics Europe report - the Facts 2021

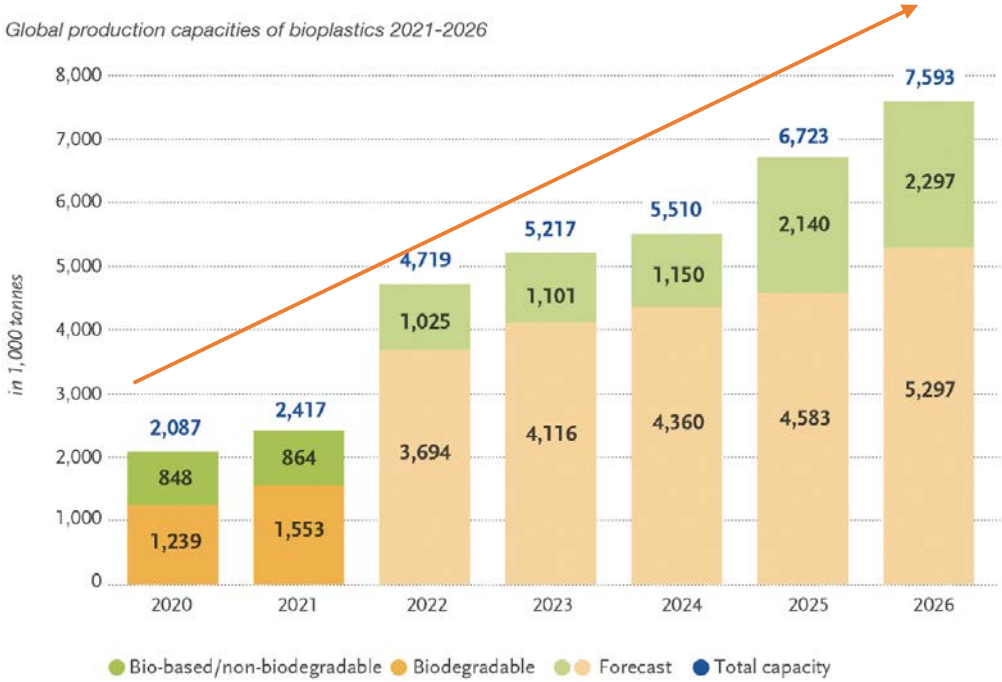
** RECYCLING: including 0.2% from chemical recycling SOURCE: Conversio Market & Strategy GmbH Above data are rounded estimations based on extrapolations of 2019 waste data for 2020.

# Poly(3-hydroxybutyrate), (PHB)

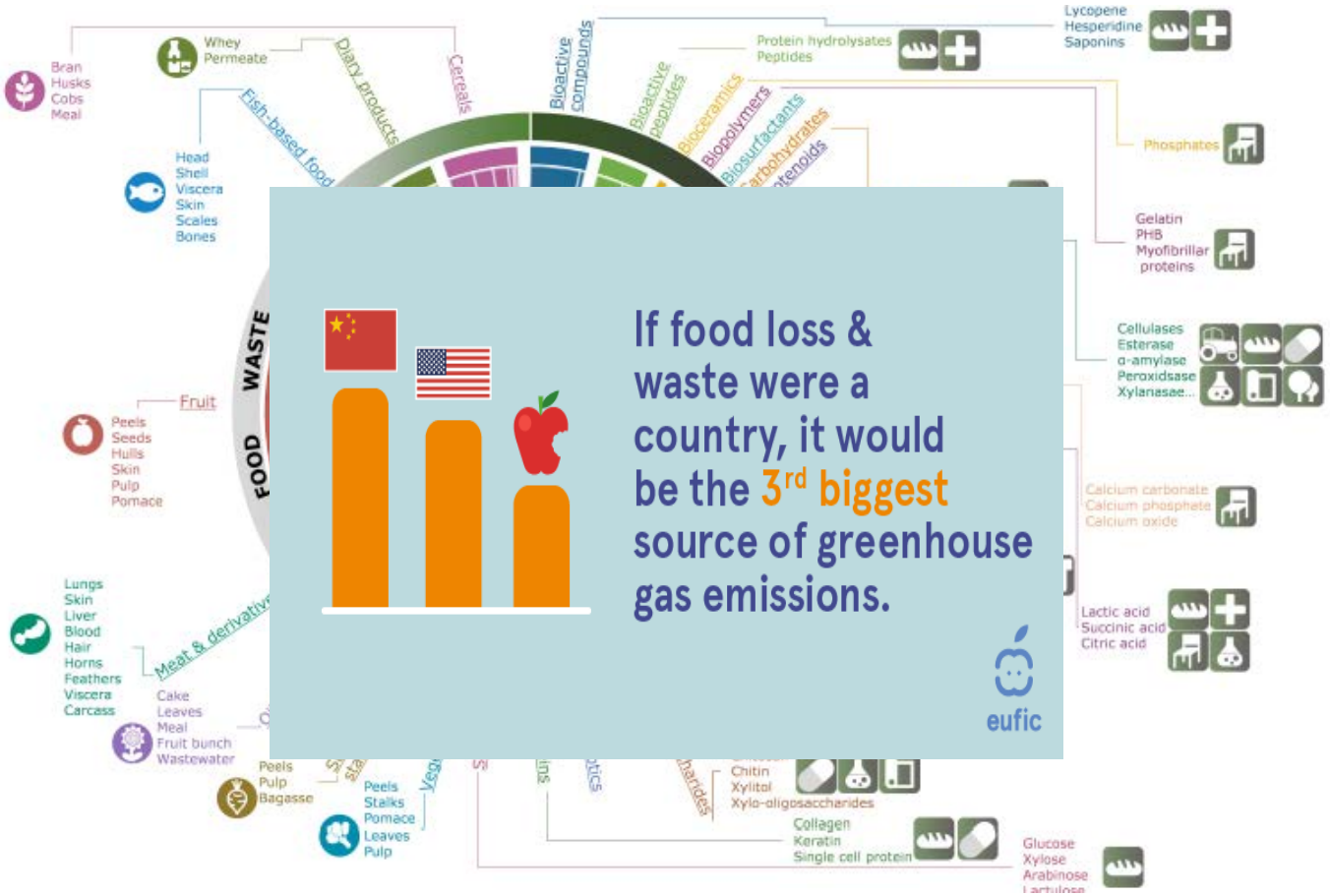



- Production cost of PHB is considerably higher compared to conventional plastics
- Future prospects are mainly focused on promoting less expensive substrates, improved fermentation strategies, and downstream processing methods, which are required for reducing production costs



# Fruit wastes




- It is estimated that approximately 1.3 billion tons of food are lost (food services and households) every year
- The environmental impacts of food waste are massive that if wasted food was a country it would be the 3rd biggest source of greenhouse gas emissions
- Where fresh fruit and vegetables contribute to 32% of the food waste generated
- EU households generate 35.3 kg of fresh fruit and vegetable waste per person per year

# PHB production by *Paraburkholderia sacchari*

**FREE SUGARS**

*Solid to liquid ratio: 1:20 (w/v) $H_20$*

Effect of the initial sugar concentration on microbial growth and PHB production

Effect of carbon to phosphorous ratio (C/P) on PHB production

Evaluation of phosphorous supplementation in the feeding solution

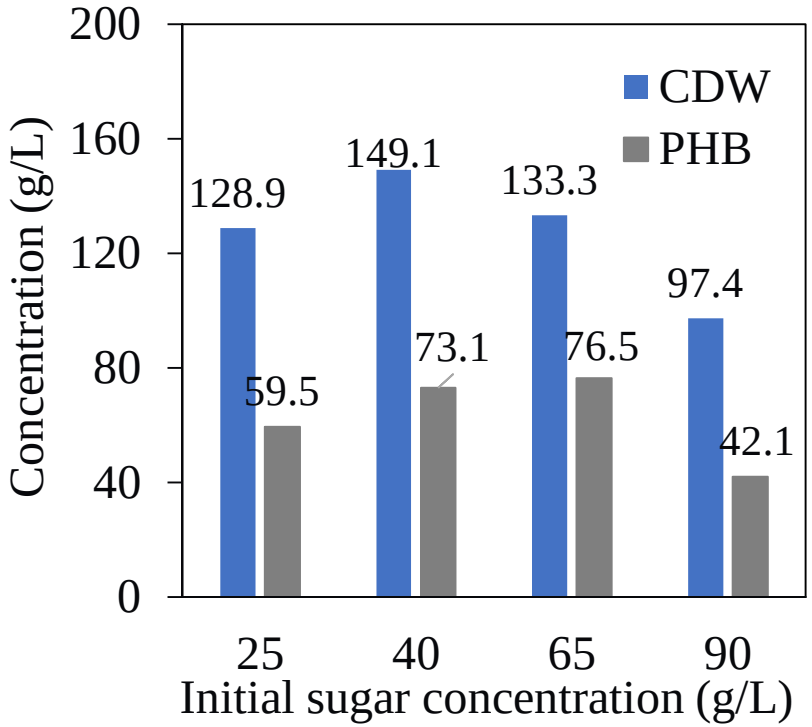
Effect of volumetric oxygen transfer coefficient ($k_La$)

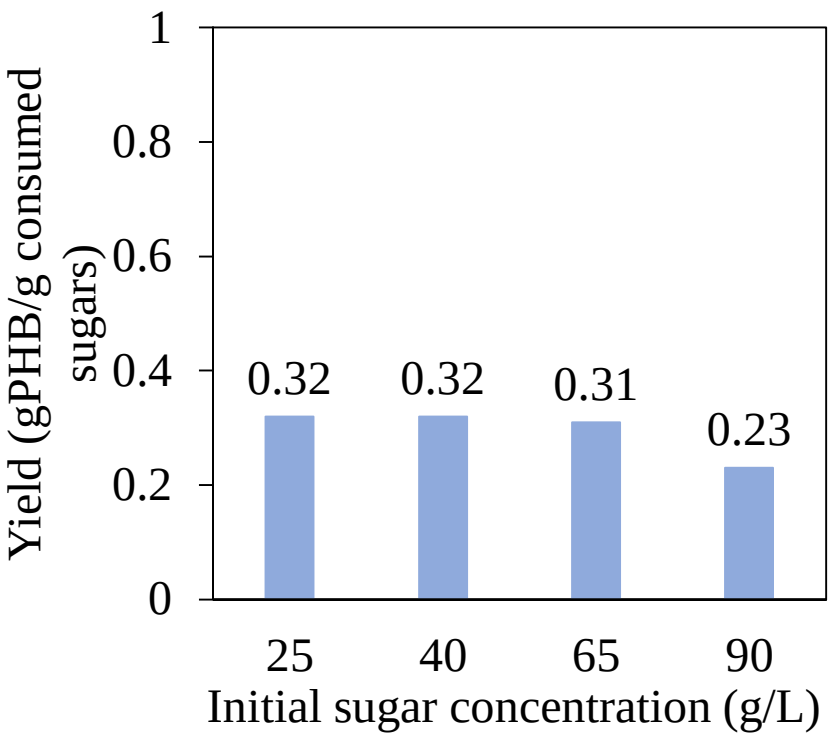
**Fed-batch fermentation**

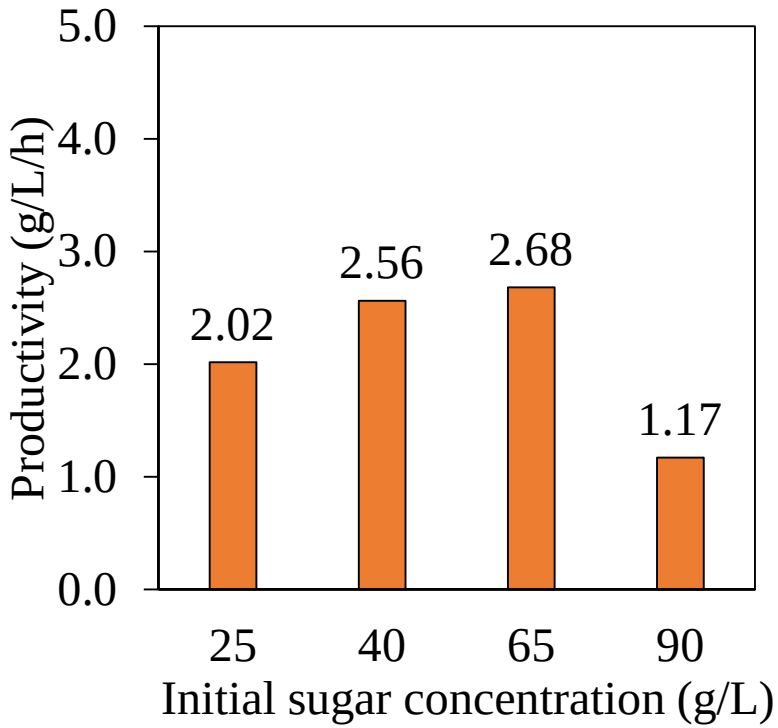
**Conditions:**

- ✓ 2 L stirred-tank reactor (Eppendorf, Bioflo120) with a working volume of 1 L
- ✓ Agitation speed: 1200 rpm
- ✓ T: 30˚C, pH: 6.8 (28% $NH_4OH$ and 2M HCl)
- ✓ Feeding solution: Free sugars
- ✓ Phosphorous limitation strategy for promoting the PHB accumulation

# Effect of the initial sugar concentration on microbial growth and PHB production

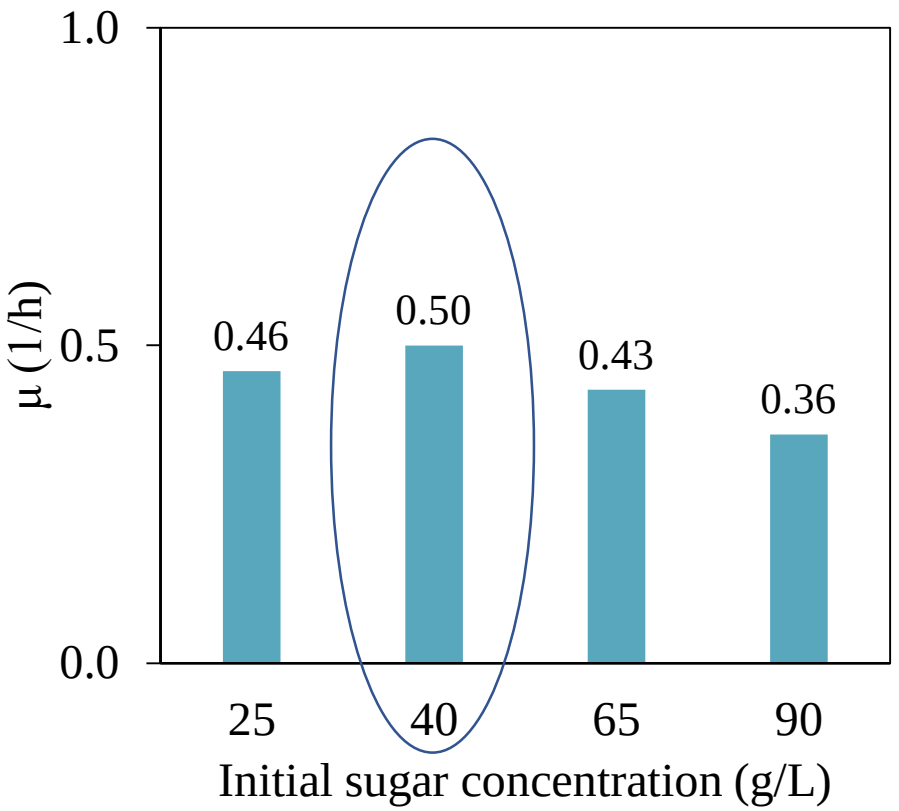


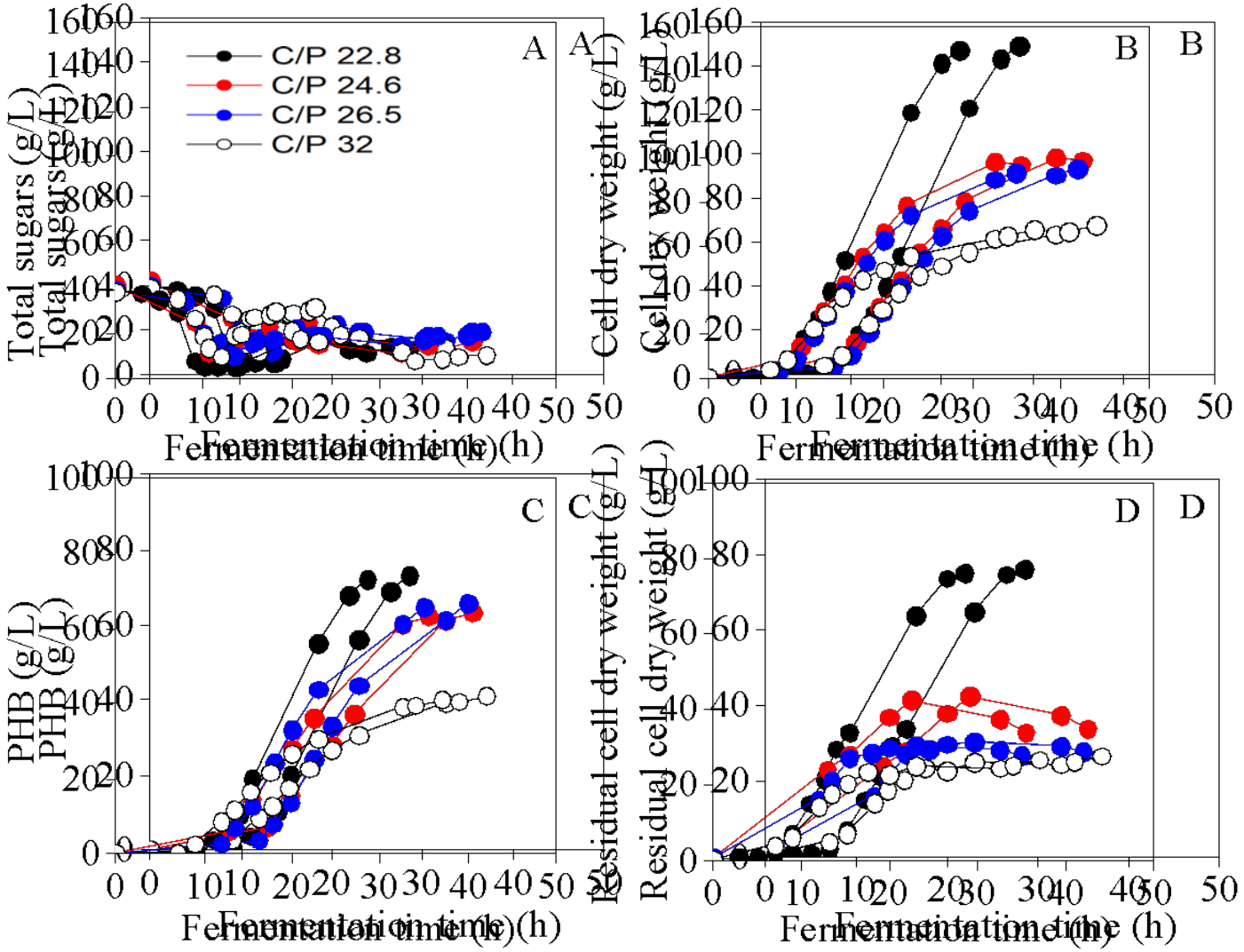

- Further, increasing the initial carbon source concentration to 90 g/L led to decreased PHB concentration, CDW, yield and productivity

# Effect of the initial sugar concentration on microbial growth and PHB production




- No growth inhibition was observed up to 65 g/L initial sugar concentration
- The highest specific growth rate was at initial sugar concentration of 40 g/L
- At higher sugar concentrations, specific growth rate was decreased

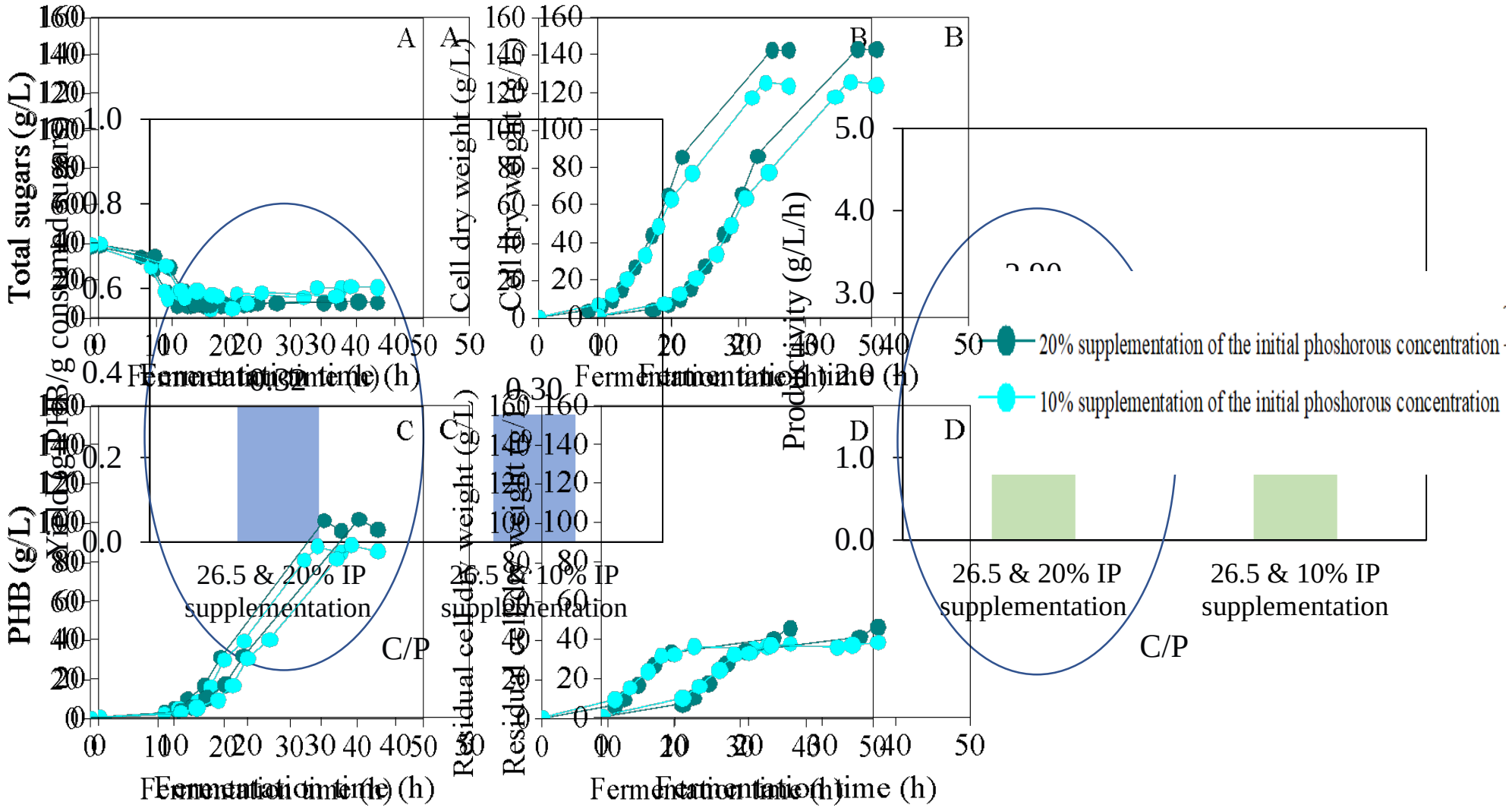
# Effect of carbon to phosphorous ratio (C/P) on PHB production



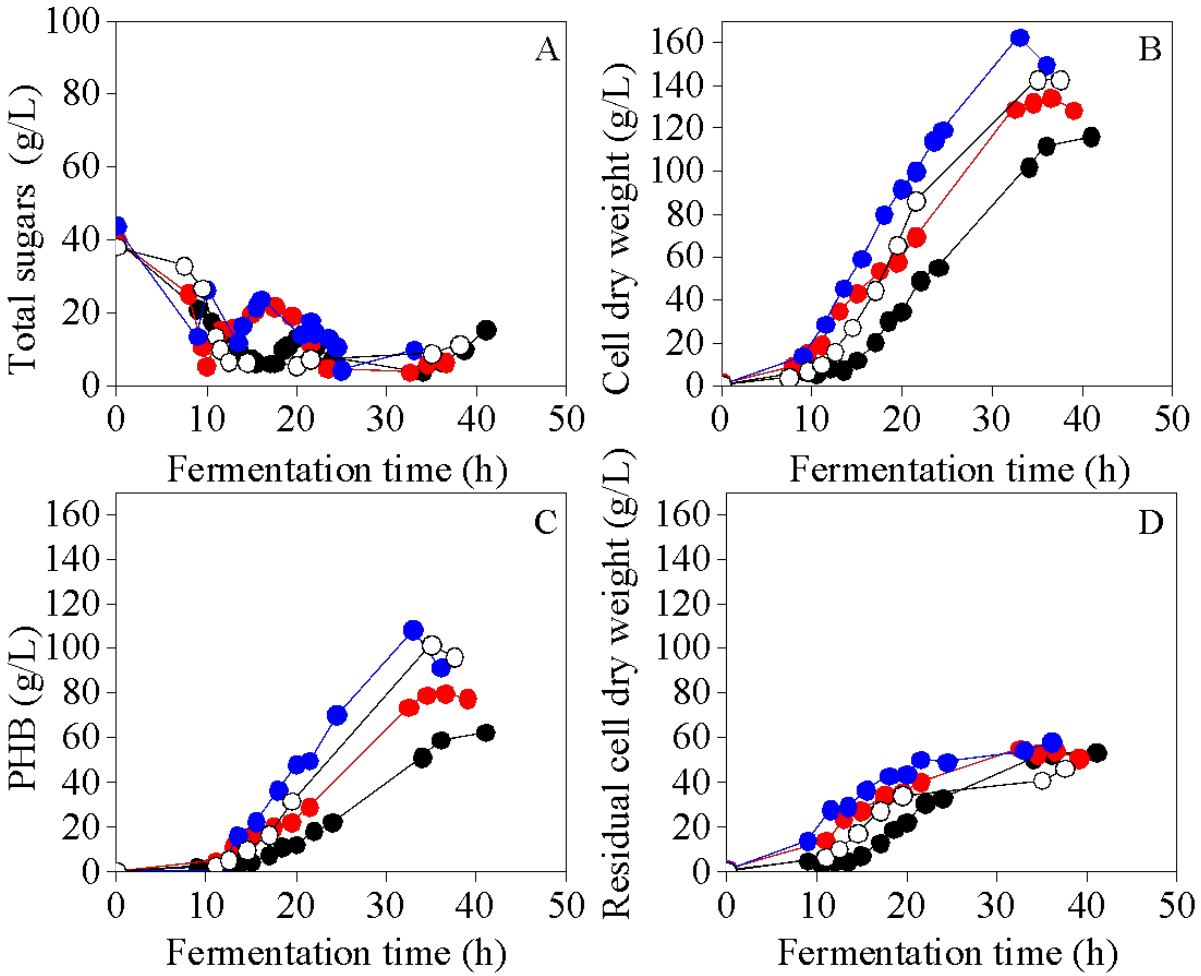

- C/P ratio was stepwise increased from 22.8 to 32, the PHB concentration and CDW gradually decreased from 73.1 g/L to 39.2 g/L and 149.1 to 63.5 g/L respectively
- Highest CDW with PHB accumulation (49%) was achieved at the lowest C/P ratio (22.8)
- The highest PHB accumulation (68%) was observed at the 26.5 with a total dry weight of 90 g/L

| C/P | Time (h) | CDW (g/L) | PHB (g/L) | PHB (%) |
|---|---|---|---|---|
| 22.85 | 28.5 | 149.1 | 73.1 | 49 |
| 24.6 | 32.5 | 98.4 | 60.9 | 62 |
| 26.5 | 32.5 | 90.5 | 61.3 | 68 |
| 32.0 | 32.5 | 63.5 | 39.2 | 62 |

# Evaluation of phosphorous supplementation in the feeding solution



# Effect of volumetric oxygen transfer coefficient ($k_La$)




| $K_la$ ($h^{-1}$) | Time (h) | CDW (g/L) | PHB (g/L) | Yield (g PHB/g consumed sugars) | Productivity (g//L/h) |
|---|---|---|---|---|---|
| 157.90 | 36.0 | 111.8 | 59.1 | 0.29 | 1.64 |
| 180.25 | 34.5 | 132.0 | 79.2 | 0.30 | 2.19 |
| 202.59 | 33.0 | 162.6 | 108.3 | 0.33 | 3.28 |
| 224.94 | 35.0 | 142.7 | 101.6 | 0.32 | 2.90 |

**$k_La$ = 224.9 $h^{-1}$**

**$k_La$ = 202.6 $h^{-1}$**

**$k_La$ = 180.2 $h^{-1}$**

**$k_La$ = 157.9 $h^{-1}$**

# Conclusions

- The highest specific growth rate observed at 40 g/L initial sugar concentration for *P. sacchari*
- C/P ratio of 26.5 resulted in the highest yield and the highest intracellular PHB content of 68%
- Phosphorous in the feeding solution improved both bacterial growth and PHB accumulation
- $k_L a$ value of 202.6 $h^{-1}$ resulted in the highest yield (0.33 g/g) and productivity (3.28 g/L/h)

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# Thank you for your attention!

**"Bioconversion of Food Industry Wastes to Biopolymers for Packaging Applications in a Biorefinery Concept - Wastes-to-Biopolymers"**

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