

# Cost reduction for biodiesel production from distillery/domestic mixed wastewater by *Rhodospiridium toruloides*

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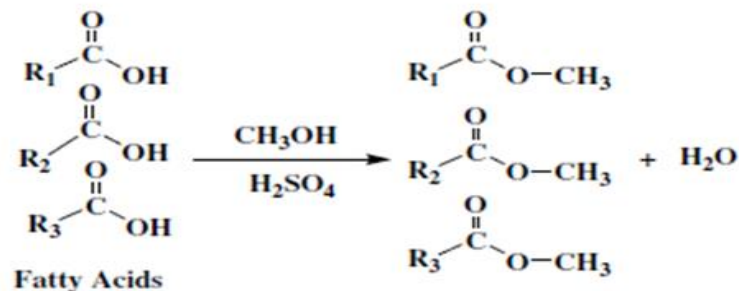
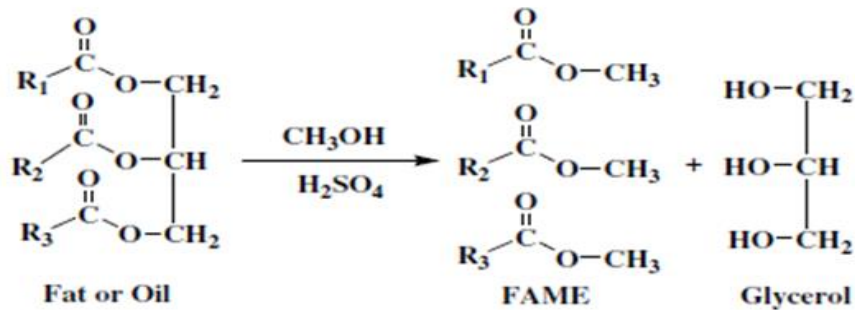
# Introduction

## ➤ Shortage of fossil fuel

- Energy crisis & High energy prices
- Environmental security



## ➤ Biodiesel: renewable & environmentally friendly



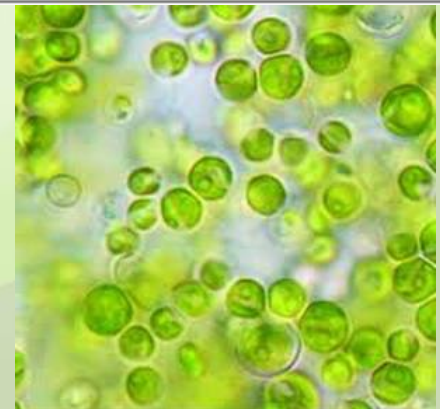
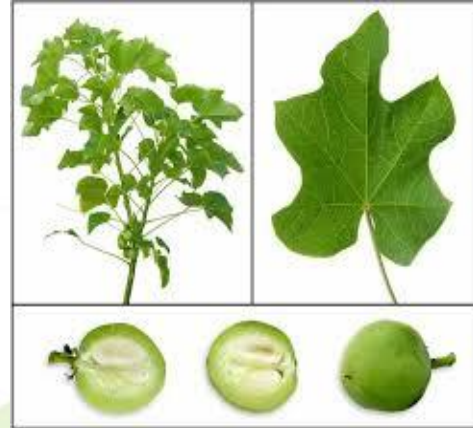
# Introduction



- Large amount of food industry wastewater generated
  - Annual wastewater generated from brewery in China: **210,000,000 tons by 2012**
- Food industry wastewater is difficult to treat
  - High COD
  - High TN and TP
  - Could lead to eutrophication and algal blooms
- Cost-effective treatment process is required

# Some Challenges in Biodiesel Industry

- ▶ 1st Generation Biodiesel—derived from vegetable oils crops (e.g., rapeseed, soybean)
- ▶ 2nd Generation Biodiesel—produced using oils from non-edible plants (e.g., Jatropha, Ricinus)
  - Crops for biodiesel production vs. Crops for food supply
  - More cropland, less forest, more green house gas
- ▶ 3rd Generation Biodiesel—processing oils from oleaginous microorganisms (e.g., Microalgae, Yeast)
  - Usage of fertilizer or external carbon source required
  - Cost of production too high



# Introduction

High density  
culture

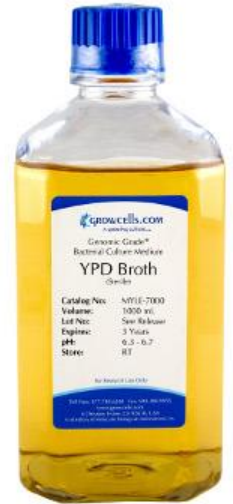


High lipid production  
in real wastewater

Preparation of high  
cell density seed  
culture



Large amount of spent  
YPD medium generated  
(80-90% of seed culture  
medium)



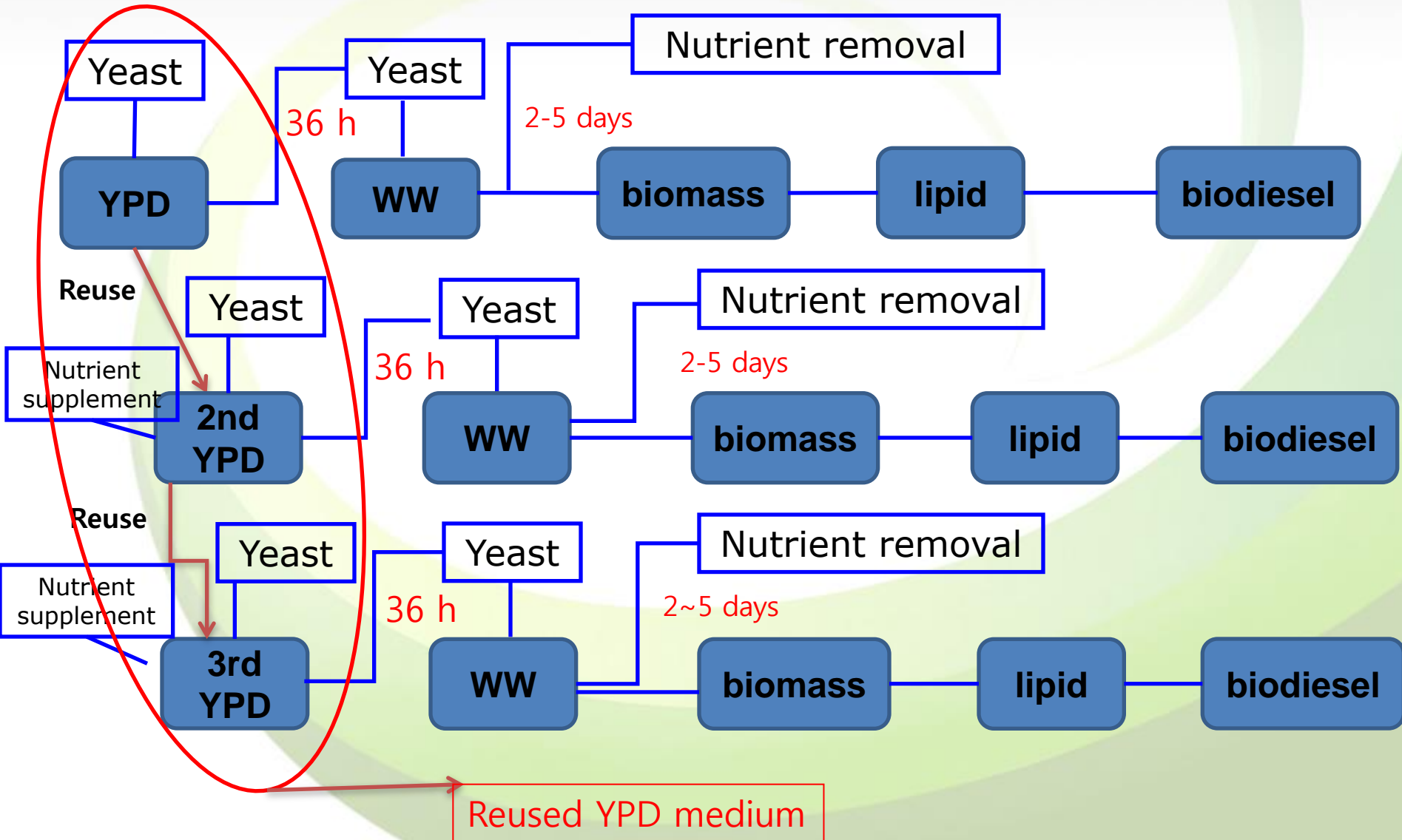
- YPD medium is rich in glucose and nutrient (Yeast extract 10 g/L, Peptone 20 g/L, Glucose 20 g/L)

# Objectives

- To explore potential of reusing spent seed culture medium
- To assess lipid productivity and removal efficiencies for organics and nutrients for cells produced from spent medium, compared to cells grown in fresh medium



# Materials and Methods



# Materials and Methods

## ➤ Wastewater

- Rice wine distillery wastewater (Foshan)
- Domestic wastewater (Macau)



## ➤ Seed Culture Medium (YPD medium)

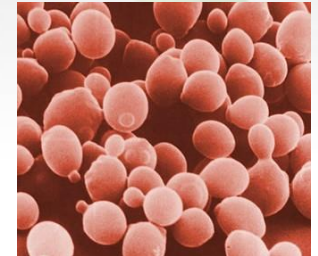
- Yeast extract 10 g/L
- Peptone 20 g/L
- Glucose 20 g/L

Characteristics of Wastewater

Wastewater	SCOD (mg/L)	TN (mg/L)	TP (mg/L)	NH <sub>3</sub> -N (mg/L)	pH
Distillery Wastewater	59,900	2,680	380	204	3.7
Domestic Wastewater	49	11	2	8	7.6
Mixed Wastewater (1:1)	29,100	1,255	179	107	3.7



# Materials and Methods

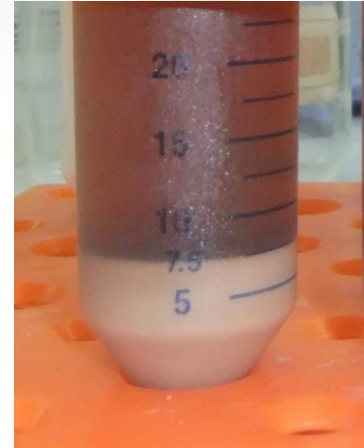


- Oleaginous Microorganisms
  - Oleaginous yeast *Rhodosporidium toruloides*
- Experimental Setup
  - Inoculated to 50 mL sterilized YPD fresh, 2<sup>nd</sup>, and 3<sup>r</sup> time used medium with or without nutrient supplement
  - Cultured at 30°C, 200 rpm, 36 h
  - Harvested by centrifuging at 4,000 rpm for 10 min
  - Dry weights of cells produced by each type of medium were measured

# Materials and Methods

## ➤ Experimental Setup

- Different kinds of seed culture were centrifuged at 4,000 rpm for 10 min to cell density of  $1.5 \times 10^9$  cells/mL
- Inoculated in 30 mL real non-sterile distillery/ domestic mixed wastewater with initial cell density of  $2 \times 10^7$  cells/mL and cultured at 30°C, 200 rpm, 5 day
- Yeast biomass was harvested by centrifuging
- COD, TN, TP, pH of supernatant measured



# Materials and Methods

## ➤ Analytical Methods

- Dry weight of yeast biomass: dried at 80°C to constant weight
- Lipid yield: acid-heat method
- COD, TN, TP: Hach method

# Results

Yeast cell yields from fresh and reused media

<b>Medium type</b>	<b>Fresh</b>	<b>Reused (2nd time)</b>		<b>Reused (3rd time)</b>	
<b>Nutrient addition (%)</b>	<b>100</b>	<b>0</b>	<b>10</b>	<b>0</b>	<b>50</b>
<b>Cell yield (g/L)</b>	<b><math>6.04 \pm 0.84</math></b>	<b><math>3.17 \pm 1.08</math></b>	<b><math>3.67 \pm 0.31</math></b>	<b><math>2.65 \pm 0.71</math></b>	<b><math>3.81 \pm 0.43</math></b>

# Results

## COD in Wastewater (mg/L)

<b>Time (day)</b>	<b>0</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Fresh YPD	29,100	6,229	3,417	2,545	1,971
2nd time used YPD	29,100	3,938	2,903	3,250	2,100
2nd time used YPD with 10% nutrient supplementation	29,100	3,963	3,283	2,455	2,286
3rd time used YPD	29,100	11,046	4,404	2,760	2,231
3rd time used YPD with 50% nutrient supplementation	29,100	11,250	3,158	2,556	2,471

# Results

## Total Nitrogen in Wastewater (mg/L)

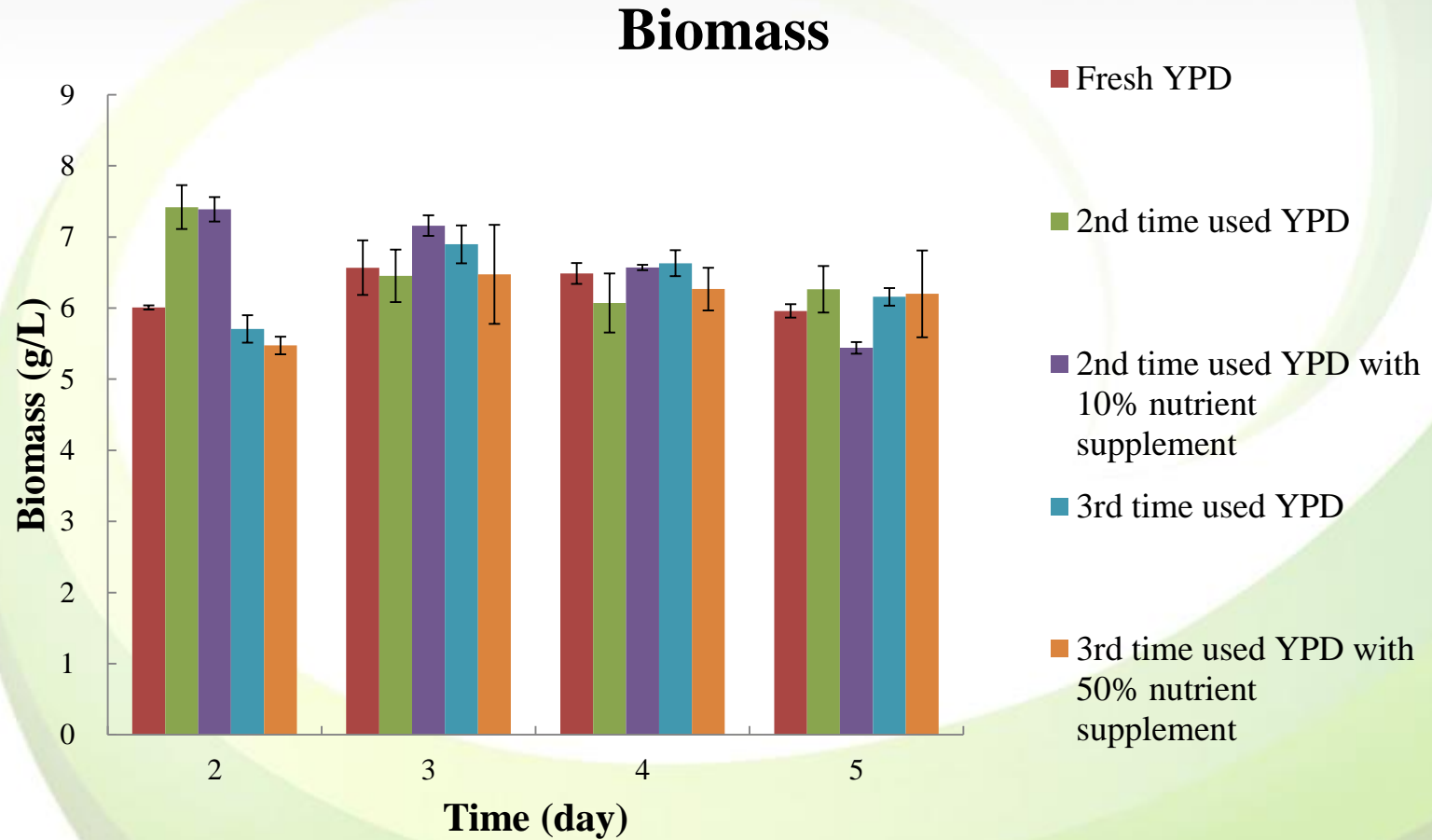
<b>Time (day)</b>	<b>0</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Fresh YPD	1,255	659	541	659	726
2nd time used YPD	1,255	618	554	709	794
2nd time used YPD with 10% nutrient supplementation	1,255	634	609	694	823
3rd time used YPD	1,255	798	624	681	675
3rd time used YPD with 50% nutrient supplementation	1,255	808	577	674	641

## Total Phosphorus in Wastewater (mg/L)

<b>Time (day)</b>	<b>0</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
Fresh YPD	179	49	33	35	41
2nd time used YPD	179	46	51	44	45
2nd time used YPD with 10% nutrient supplementation	179	50	33	46	47
3rd time used YPD	179	64	39	31	36
3rd time used YPD with 50% nutrient supplementation	179	76	43	34	38

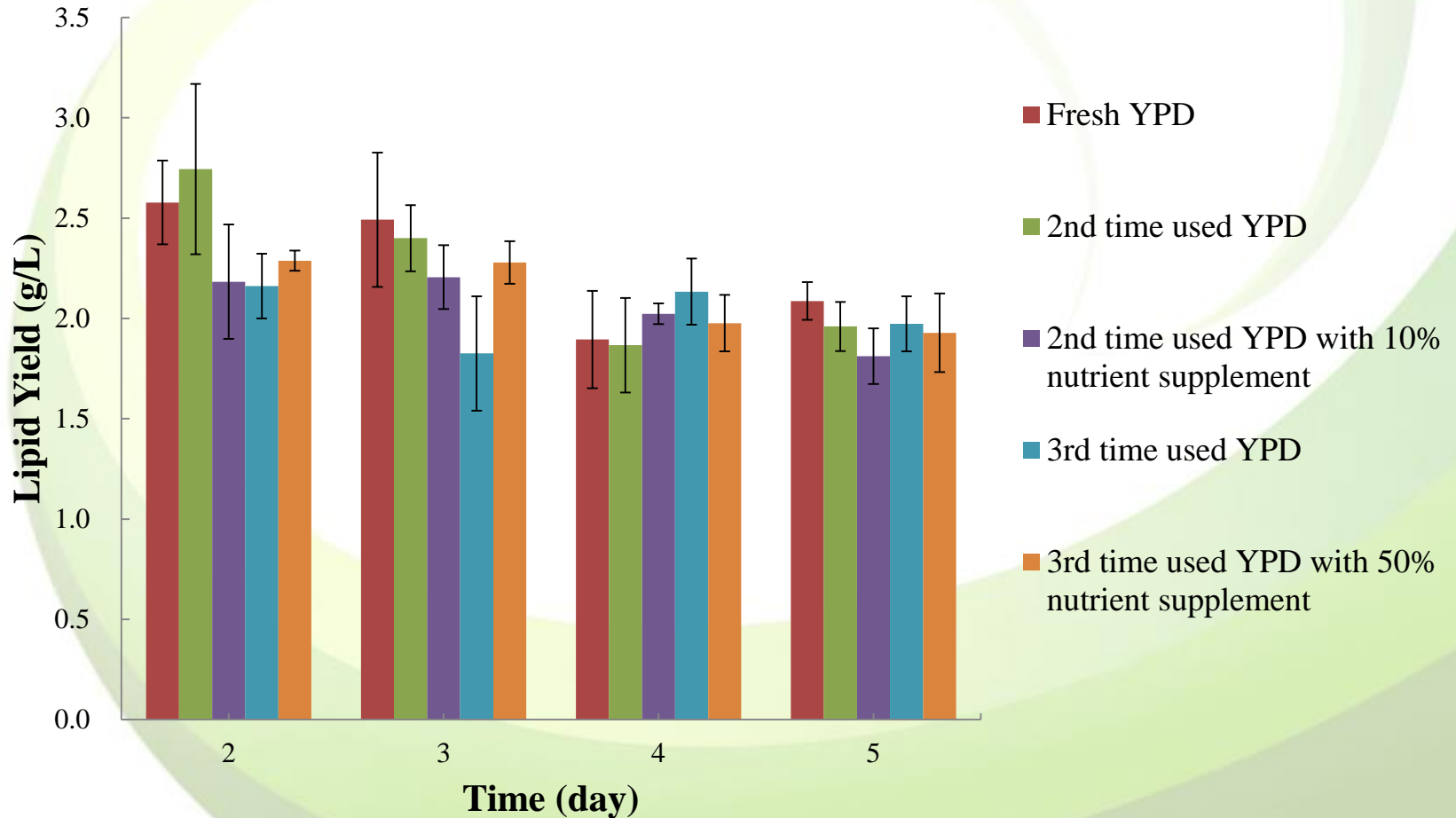


# Results



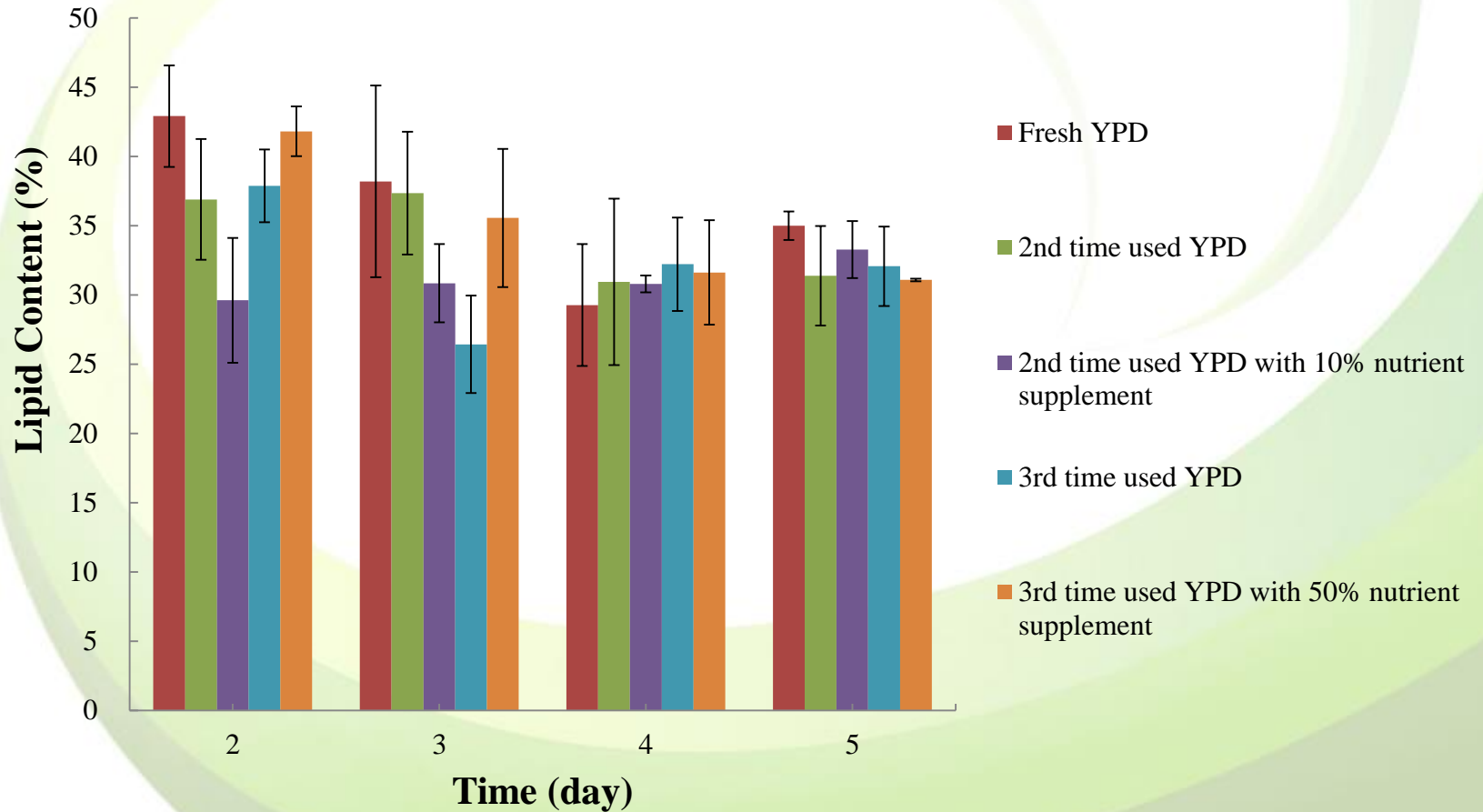
# Results

## Lipid Yield



# Results

## Lipid Content (%)



# Results

## Cost for producing amount of cells produced from 50 L fresh medium

	Amount of spent medium generated by 1 L fresh medium (mL)	Amount of cells produced by spent medium from 1 L fresh medium (g/L)	Amount of cells produced by 1 L medium (g/L)	Medium amount required to produce the amount of cells produced by 50 L fresh medium (L)	Total cost of materials for medium (USD)	Price for glucose (USD/kg)	Price for peptone (USD/kg)	Price for yeast extract (USD/kg)
Use of fresh medium only	800	0	6	50	389	9.8	270	218.5
Use of fresh and 2nd time used medium	800	2.4	8.4	35.7	278	9.8	270	218.5

# Conclusion

- The **spent YPD medium** generated while in preparation of seed culture with **high cell density** was **successfully reused for the 2nd time** without further addition of nutrient.
- The **cells** produced by **reused medium** showed **remarkable biomass** production with **similar lipid productivity** and **higher removal efficiencies** for **organics** and **nutrients** in real non-sterile mixed wastewater in **shorter cultivation time** compared to the fresh medium.
- **Cells** produced from the **3rd time used medium** did not show a **good performance** regardless of nutrient supplementation.
- **Reutilization of spent medium** showed **high potential** in the **reduction** of **material cost** for microbial **lipid production** process.

# Acknowledgements

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