Evaluation of lipid productivity of *Rhodosporidium toruloides* under non-sterile condition from a mixture of distillery and domestic wastewater

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Fossil fuels vs. Renewable energy

- **Shortage of fossil fuel:**
  - **World liquid fuels: 87 million barrels/day (2010) to 119 million/day (2040)**
  - **Unsustainable: reserves fast declining and greenhouse gas emission**
  - **Anthropogenic emission of carbon: 20 billion tons/year**

- **Renewable energy:**
  - **Solar, wind, hydro, biomass, or geothermal**
  - **19% of total global energy consumption in 2012**
  - **30-45% in 2050: fast growing global investment to $400-500 billion/year**

Some Challenges in Biodiesel Industry

- **1st Generation Biodiesel—vegetable oils crops**

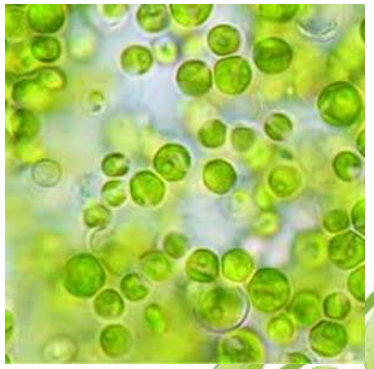
  Crops for biodiesel production vs. Crops for food supply

- **2nd Generation Biodiesel—oils from non-edible plants**

  Not mature for industrial purposes

- **3rd Generation Biodiesel—oleaginous microorganisms**

  Production cost still too high



Food Industry Wastewater

- **Annual wastewater generated from brewery in China:**

210 million tons by 2012

- **Cost investment for wastewater: $20.9 billion (2019)**

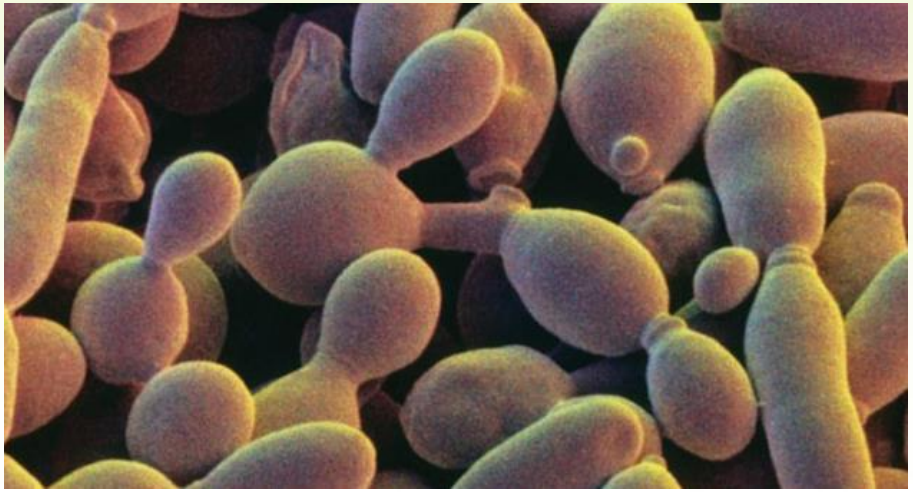
- **Food industry wastewater difficult to treat:**

  - **High COD**
  - **High TN and TP**

- **Need for more cost-effective treatment process**

Oleaginous Microbes

Why yeast?

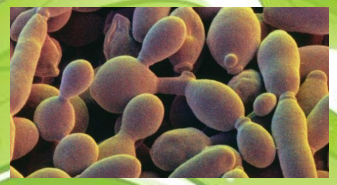


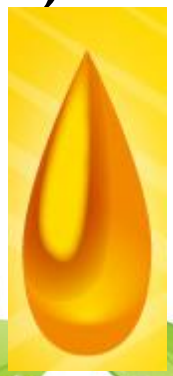
- ✓ **Long cultivation times for microalgae (6-30 days)**
- ✓ **Oleaginous yeast with remarkable lipid production**
- ✓ **Shorter incubation time (30-144 h)**

Objectives

- To treat food industry wastewater (mixture of distillery and domestic) effectively

- To produce microbial lipid/biodiesel from wastewater under non-sterile condition (effect of indigenous organisms)





Experimental

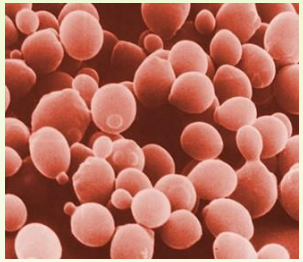
➢ Wastewater

- Rice wine distillery wastewater (Foshan, China)
- Domestic wastewater (Macau SAR, China)

Characteristics of Wastewater

Wastewater	SCOD (mg/L)	TN (mg/L)	TP (mg/L)	NH_3-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

Experimental



Rhodosporidium toruloides AS 2.1389
(from China General Microbiological Culture Collection Center)

Yeast

YPD → Yeast cells

30ºC, 200 rpm, 36 h

30ºC, 200 rpm, 5 days

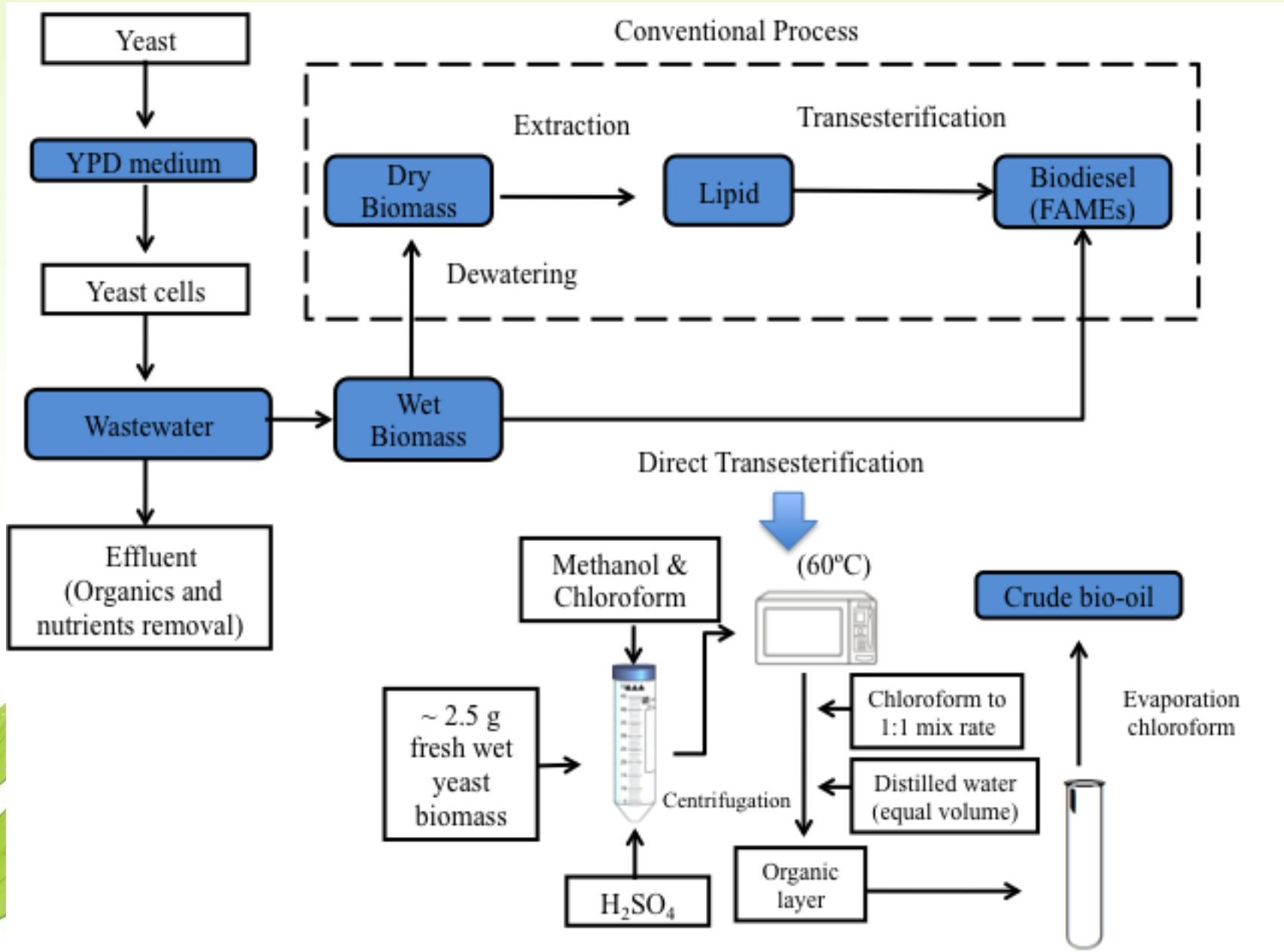
Extraction

Wastewater → Biomass → Lipid → Biodiesel

Effluent
(Organic matters
&
Nutrients
removals)

Transesterification

Experimental flow

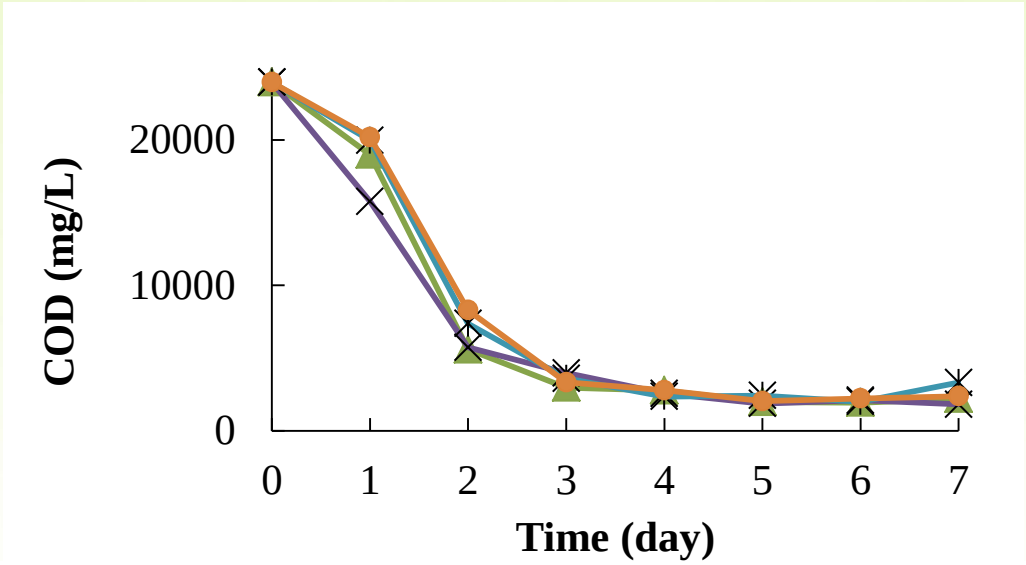


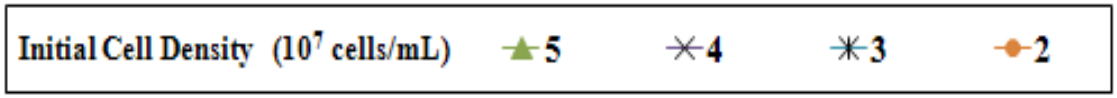
Experimental

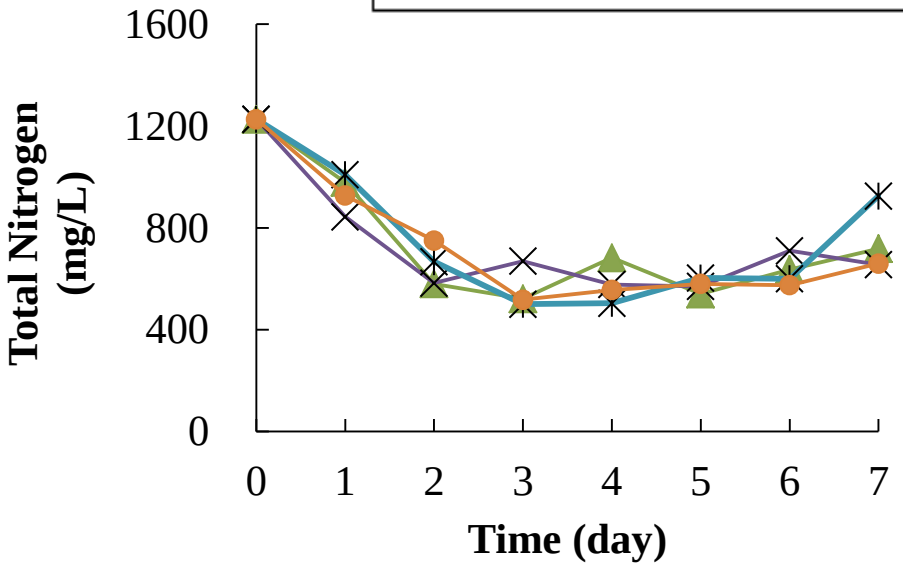
➢ Analytical Methods

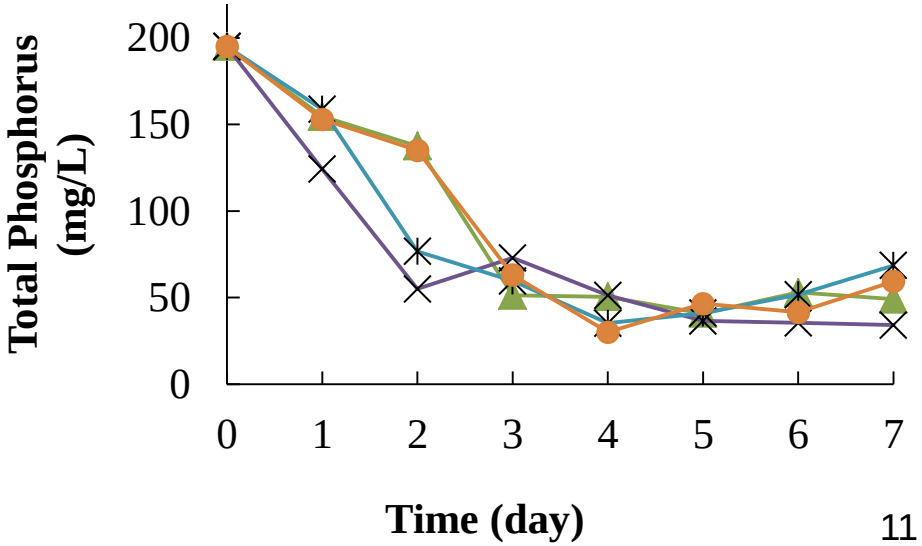
- **Dry weight of yeast biomass**
- **Lipid yield: acid-heat method**
- **COD, TN, TP: Hach method**
- **FAMEs :**
  - ✓ **Dissolved in 5 mL hexane**
  - ✓ **Gas chromatography with flame ionization detector**

Effect of initial cell density (-organics/nutrients removal-)

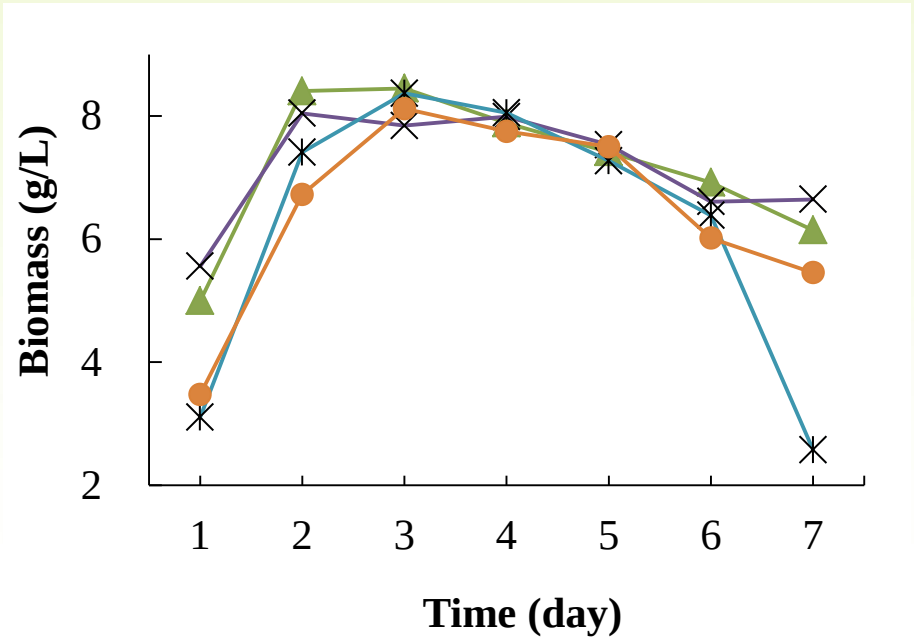


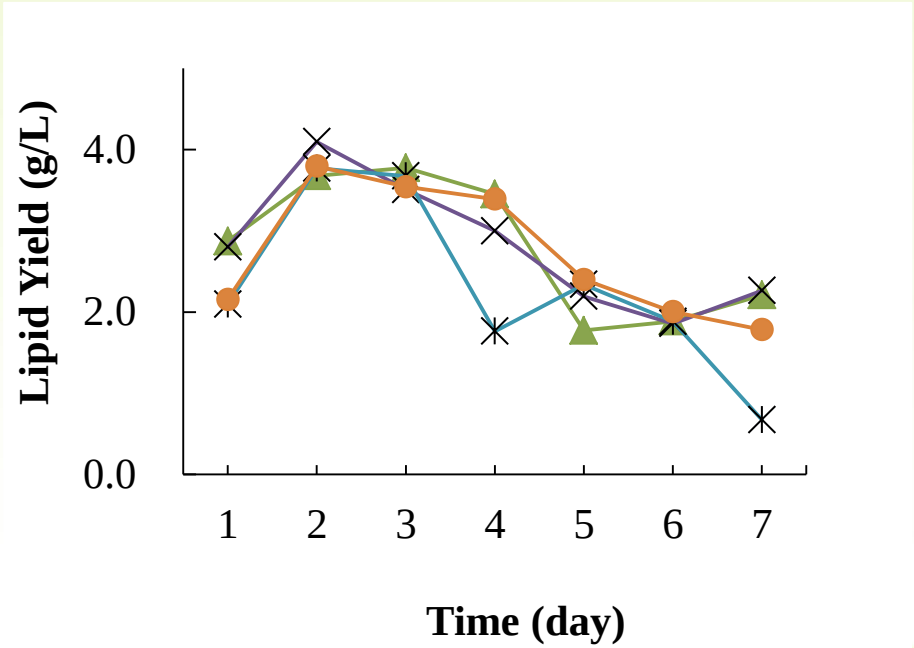






Effect of initial cell density (-biomass and lipid yield-)





Initial Cell Density (10^7 cells/mL) 5 4 3 2

Results

Contribution of indigenous organisms to specific removal of organics/nutrients and specific lipid production from mixed wastewater after 3 days of cultivation

Microorganism	Cell density (cell/mL) x 10^8	Wastewater	SCOD:TN:TP	Specific COD removal*	Specific TN removal*	Specific TP removal*	Specific Biomass**	Specific lipid yield**	Specific lipid content***
R. Toruloides AS2.1389	0.20	Mixed distillery wastewater (Sterile)	249:7:1	25.61	0.34	0.08	7.10	2.89	0.42
R. Toruloides AS2.1389	0.20	Mixed distillery wastewater (Non-sterile)	249:7:1	22.34	0.30	0.06	6.04	2.33	0.30
Indigenous organisms	0.75	Mixed distillery wastewater (Non-sterile)	249:7:1	8.42	0.14	0.03	1.73	0.36	0.06

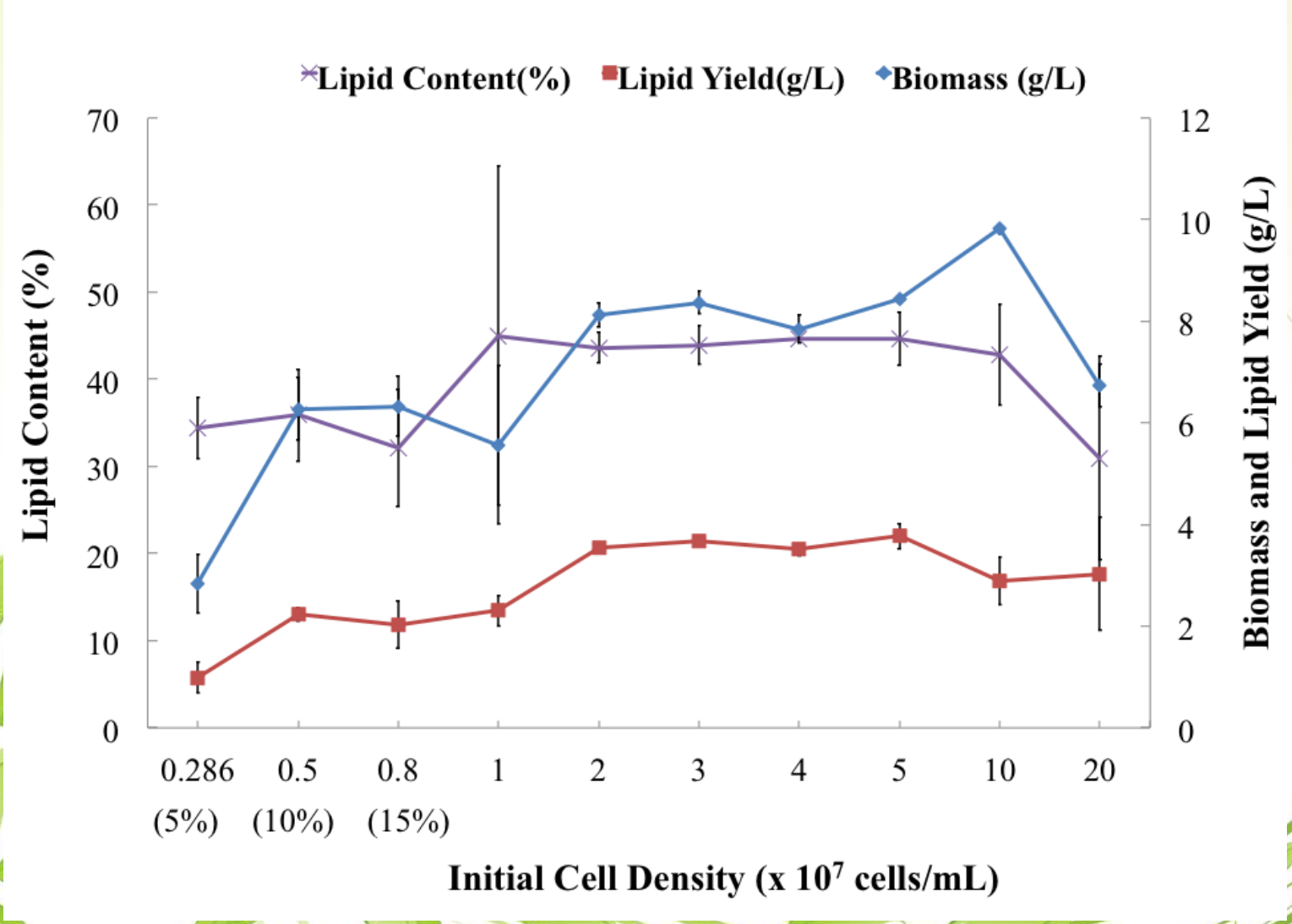
* $(mg/L\ cell^{-1}\ h^{-1}) \times 10^{-6}$

** $(g/L\ cell^{-1}\ h^{-1}) \times 10^{-9}$

*** $(g/L\ lipid/g/L\ biomass\ cell^{-1}\ h^{-1}) \times 10^{-9}$

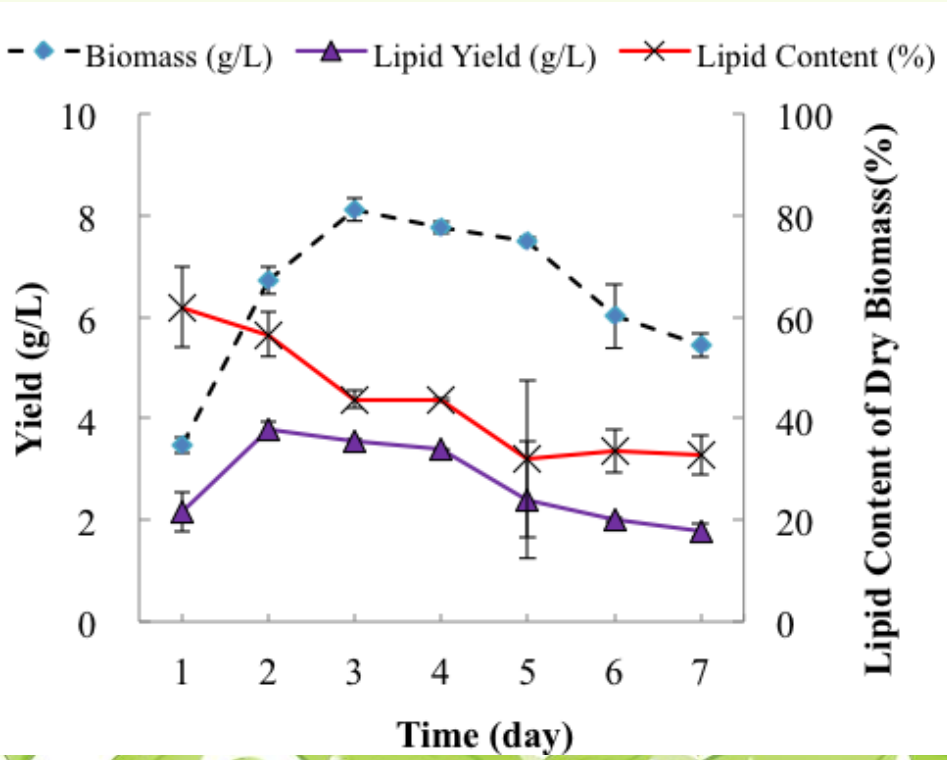
Results

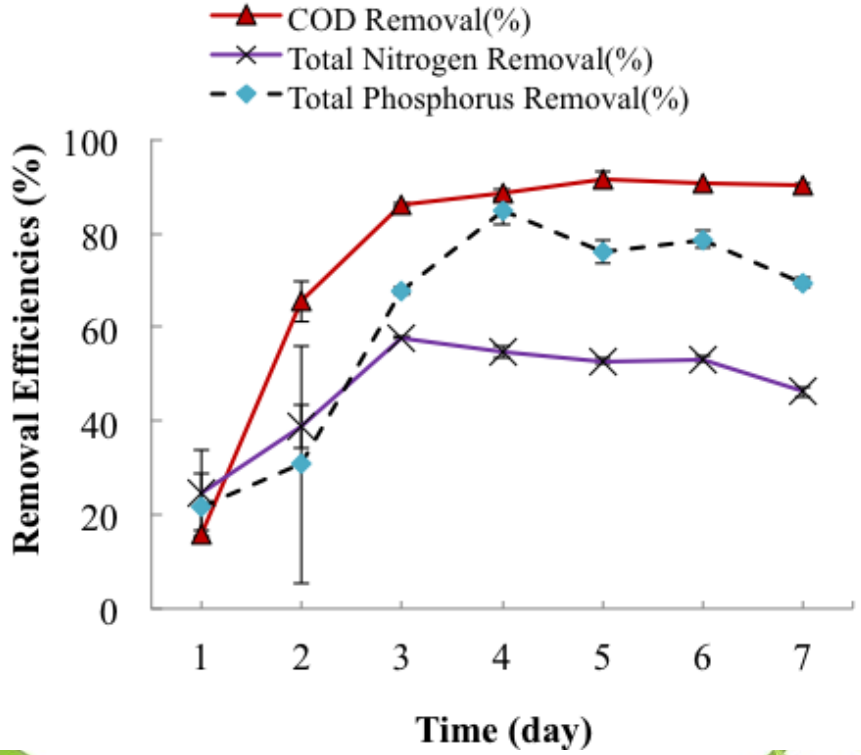
Effects of initial cell densities on lipid production in mixed wastewater under non-sterile conditions in 3-day cultivation



Results

Lipid production and organics/nutrients removal at initial cell density of 2×10^7 cells mL^{-1}





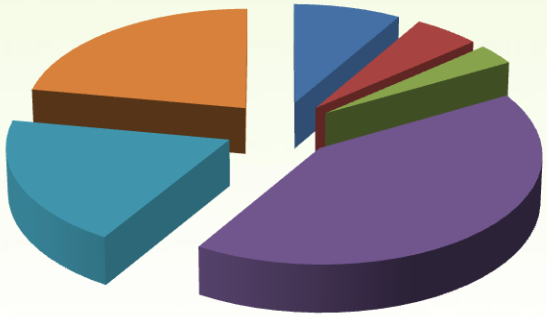
Results

Direct transesterification

Factors / Test NO.	Solvent/ biomass rate (mL/g)	Methanol and chloroform mix ratio	H_2SO_4 (%)	Reaction time (min)	FAMEs/Lipid conversion (%)	Mean FAMEs (mg/L WW)
1	8	1:0	2	10	31.53	204
2	8	1:1	4	20	37.33	241
3	8	2:1	6	30	55.99	362
4	16	1:0	4	30	37.19	240
5	16	1:1	6	10	71.12	460
6	16	2:1	2	20	42.39	274
7	24	1:0	6	20	53.06	343
8	24	1:1	2	30	53.36	345
9	24	2:1	4	10	44.60	288

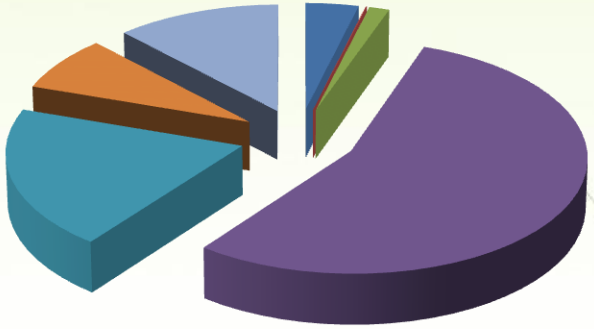
Results

Biodiesel from yeast



- Methyl Palmitate(%, C16:0)
- Methyl Palmitoleate(16:1, %)
- Methyl strearate (C18:0) (%)
- Methyl oleate (C18:1,%)
- Methyl Linoleate(C18:2,%)
- Other FAMEs (%)

Biodiesel from rapeseed



- Methyl Palmitate(%, C16:0)
- Methyl Palmitoleate(16:1, %)
- Methyl strearate (C18:0) (%)
- Methyl oleate (C18:1,%)
- Methyl Linoleate(C18:2,%)
- Linoleic acid methyl ester (C18:3,%)
- Other FAMEs (%)

Results

Comparison with other transesterification methods

Method	Temperature (℃)	Reaction time	Applied to wet biomass (Y/N)	Dewatering process required (Y/N)	Conversion (%)	Reference
Conventional	117	3 h	N	Y	≈90	Lotero et al., 2005
In Situ Transesterification	70	20 h	N	Y	98	Liu and Zhao, 2007
	120	2 h	Y	N	92	Cao et al., 2013
Microwave assisted Direct Transesterification	60	10 min	Y	N	70	This work

Conclusions

- **Generating renewable energy (biodiesel) while treating high strength wastewater without addition of external nutrient/fertilizer**
- **High removal of organic matters and nutrients (P)**
- **Applicable to non-sterile wastewater without interference of indigenous microorganisms**
- **High lipid production at relatively short time**
- **Microwave-assisted transesterification process as a promising technology with lower energy consumption**

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