Bioconversion of Fruit Juice Industry Wastewater into Single-Cell Protein using Purple Non-Sulfur Bacteria

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Introduction

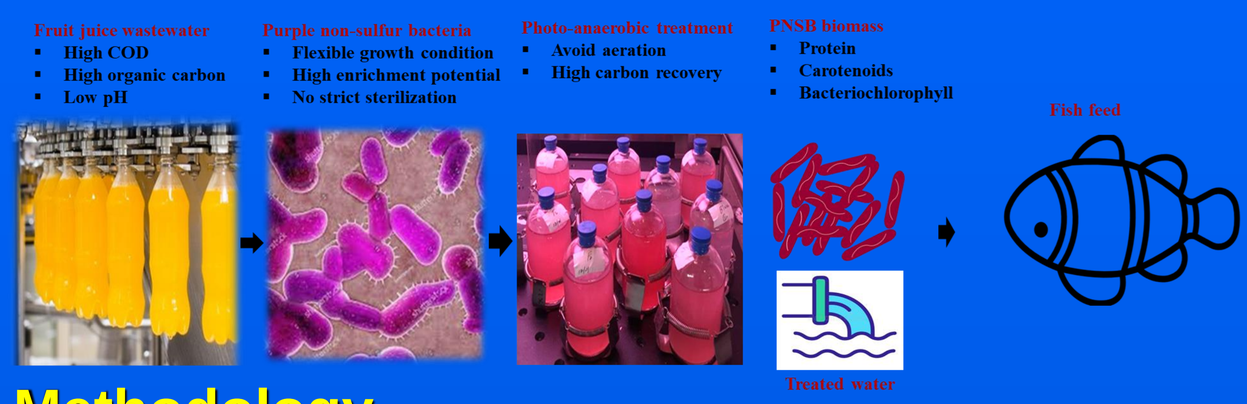
Fruit juice industry (FJW) produces a large volume of wastewater that needs to be treated before disposing into the environment to protect ecological health.

Conventional wastewater treatment techniques are energy intensive and generate large sludge volume. Anaerobic treatment coupled with resource recovery can off-set the treatment cost. Purple non-sulfur bacteria (PNSB) can be considered as promising mediators to treat FJW and recover resources from the wastewaters.

PNSB can use complex organic materials, grow in imbalanced nutrient conditions, and show high enrichment in mixed microbial culture without strict culture control and strain maintenance requirements. PNSB biomass contains value-added bio-molecules such as carotenoids, bacteriochlorophyll, and essential amino acids, which improve the nutritional value of single cell protein (SCP).

This study aims to explore the potential of PNSB to treat FJW and characterization of their biomass to assess SCP quality. Two types of juice wastewaters (i) citrus juice (CJW) and (ii) mixed juice (MJW) were used at different pH values and their effects on PNSB growth, carbon removal, biomass productivity, and SCP quality have been investigated.

Research scheme



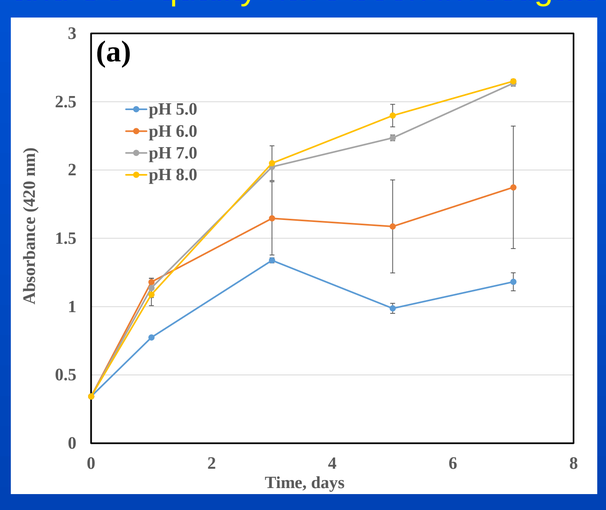
Methodology

- Real FJW was collected and characterized
- Minerals, vitamins were added as per ATCC-2672 media composition
- pH was adjusted to 5.0, 6.0, 7.0, and 8.0
- Mixed culture of PNSB was used as inoculum and bottles cultured in illuminated shaking incubator
- Growth was measured using 420 nm absorbance
- 16S microbial diversity analysed by QIIME

Table 1: Characterization of fruit juice industry wastewater

Parameters	Unit	Type of juice wastewater	
		Citrus (CJW)	Mixed (MJW)
COD(soluble)	$mg.L^{-1}$	5710 ± 28	5060 ± 14
COD(total)	$mg.L^{-1}$	6730 ± 141	5590 ± 99
TOC	$mg.L^{-1}$	2564 ± 3	2560 ± 34
TSS	$mg.L^{-1}$	500 ± 18	271 ± 13
pH	–	4.12 ± 0.05	3.85 ± 0.03
Conductivity	$\mu S.cm^{-1}$	548	478
ORP	mv	126	140

Results & Discussion



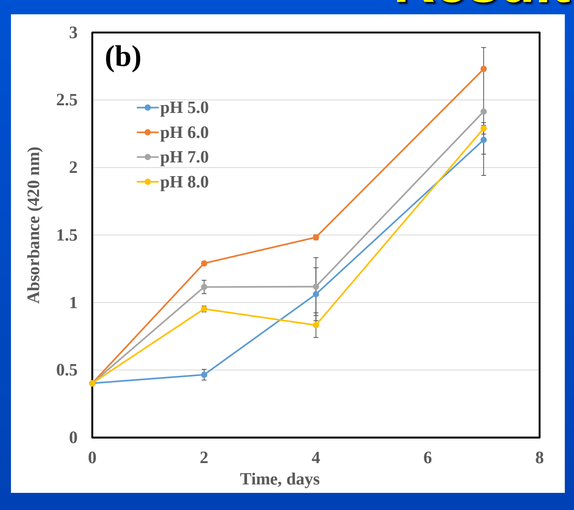
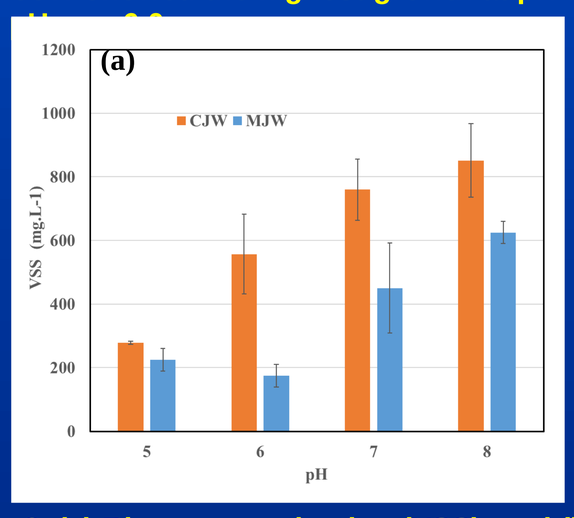


Fig 1. Growth of PNSB by 420 nm absorbance in (a) CJW and (b) MJW at different pH.

- **CJW showed the highest growth at pH 7.0 and 8.0, whereas in MJW, the optimum**



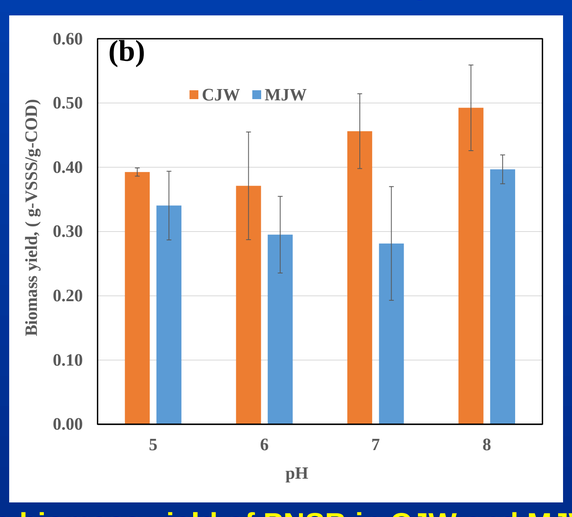
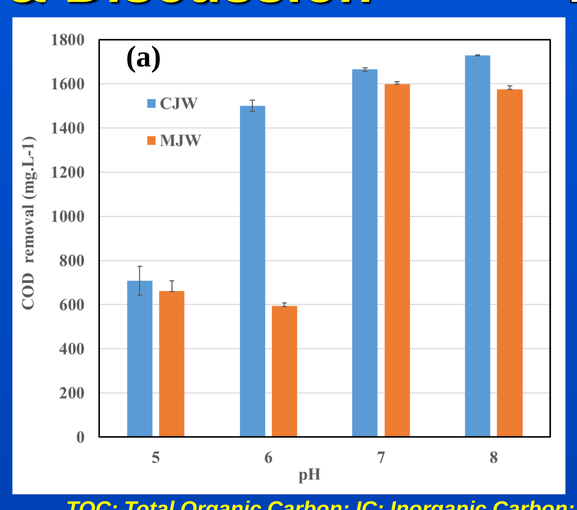


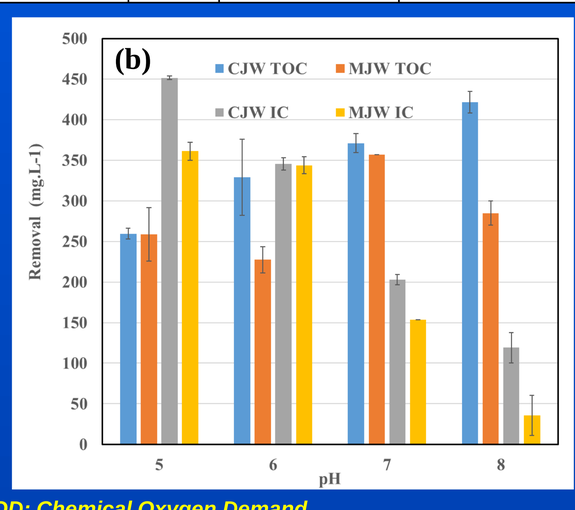
Fig 3. (a) Biomass production (VSS) and (b) the biomass yield of PNSB in CJW and MJW

- **VSS increased with an increase in the pH; the biomass yield was almost same at all pH treatment (p>0.05)**

Table 2: Biochemical composition of PNSB biomass

Parameter	Type of wastewater	
	CJW	MJW
Protein (Lowry assay), %	48 ±1	40 ± 3
Crude protein, %	64.5 ± 2.1	60 ± 3.8
Carotenoids, $\mu g.g^{-1}$	1354 ± 64	1558 ± 218
Bacteriochlorophyll, $\mu g.g^{-1}$	2097 ± 110	3100 ± 614
CHN		
Carbon, %	49.8 ± 2.3	52.7 ± 4.0
Hydrogen, %	7.53 ± 0.21	8.1±1.2
Nitrogen, %	11.9 ± 1.1	13.14 ± 0.9
C/N value	4.20 ± 0.22	4.0 ± 0.22





TOC: Total Organic Carbon; IC: Inorganic Carbon; COD: Chemical Oxygen Demand

Fig 2. (a) COD removal (b) TOC and IC removal by the PNSB in CJW and MJW

- **Both treatments i.e. CJW and MJW, showed higher COD removal at pH 7.0 and pH 8.0 than the other pH values. TOC removal increased with an increase in pH, whereas IC removal decreased with pH increase.**

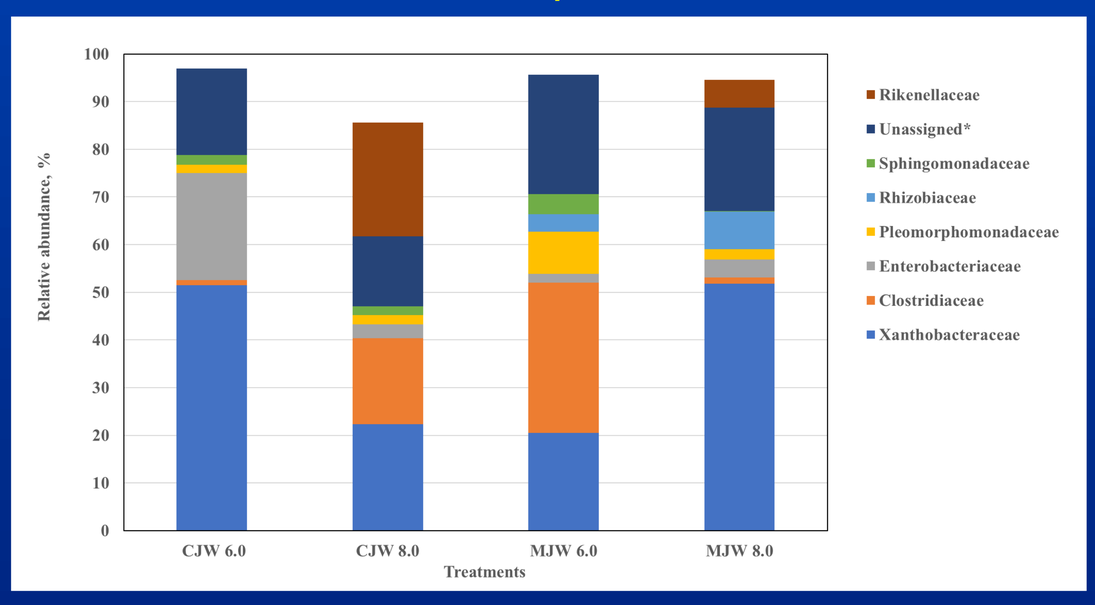


Fig 4. Relative microbial abundance at family level in CJW and MJW at pH 6.0 and 8.0. The microbial population showing >1% abundance were derived from the relative frequency barplot (up to 5 taxonomic levels) using QIIME2view. MiDAS 4.8.1 database was used to know microbial taxonomy up to genus level .

- **Xanthobacteraceae family that includes PNSB genera (*Rhodoplanes* and *Rhodopseudomonas*) showed 51% abundance in CJW at pH 6.0. MJW showed relative abundance of 51% Xanthobacteraceae at pH 8.0. 31% abundance of Clostridiaceae (nitrogen fixers) was observed in MJW at pH 6.0.**

Conclusions

PNSB can utilize juice industry wastewater as a substrate. They efficiently remove COD, organic carbon, and produce biomass (VSS). PNSB biomass contains a reasonable amount of cellular protein and photopigments that can be used as aquaculture feed and for other bio-refinery applications. The substrate utilization of PNSB depends on pH. pH 8.0 showed high treatment performance and biomass productivity. The microbial diversity analysis showed that PNSB potentially comprised up to 51% of the microbes in the culture.

The Acknowledgements

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