Influence of nitrogen on the simultaneous treatment of fuel synthesis wastewater and PNSB biofilm formation for resource recovery.

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CORFU 2022 Conference
15-18 June 2022

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Outline

- **Introduction**
- **Objective**
- **Motivation/Novelty**
- **Materials and Methods**

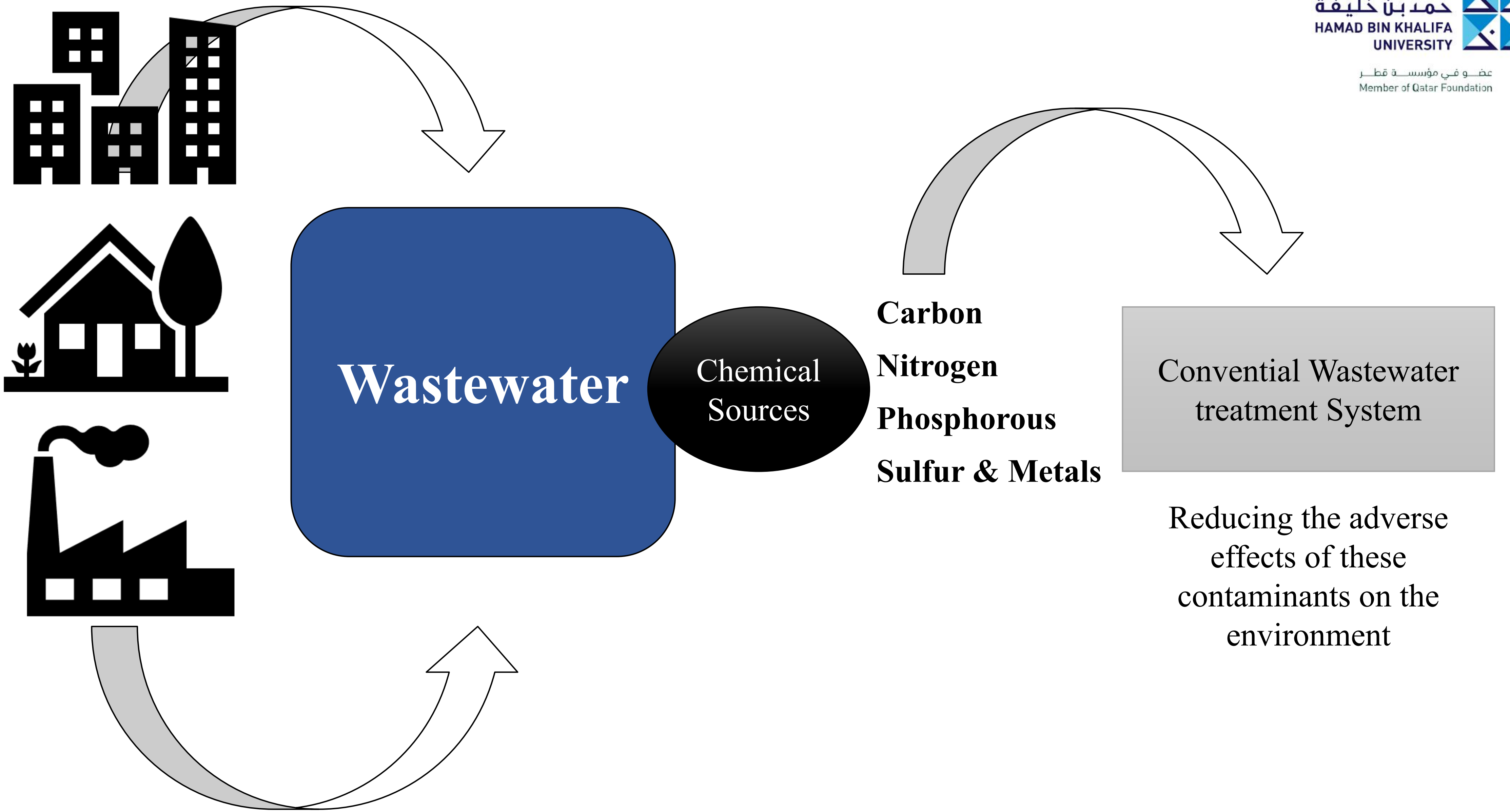
- Biofilm photobioreactor, culture condition, microorganism and growth media
- Determination of PNSB biomass from suspended and biofilm culture
- Analysis of effluent
- Determination of hydrophobicity
- Extraction of extracellular polymeric substance (EPS) and determination of EPS - polysaccharides (PS) and EPS - protein (PN)
- Extraction and determination of PNSB carotenoids and bacteriochlorophyll
- Extraction and determination of single cell protein (SCP)
- Extraction and determination of polyhydroxyalkanoates (PHAs)

- **Results & Discussion**

- Effect of nitrogen on PNSB biofilm formation and wastewater treatment
- Effect of nitrogen on hydrophobicity, EPS-PS and EPS-PN
- Effect of nitrogen on single cell protein production
- Effect of nitrogen on PHAs Production

- **Conclusion**

Introduction



Introduction

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Wastewater

Resource recovery

Organism

Reuse

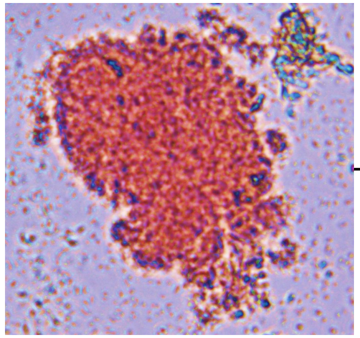
Gaseous Products

Methane, Hydrogen etc.

Biomass

Carotenoids
Bacteriochlorophyll
Single cell protein
PHAs
Coenzymes – Q10

PNSB



Versatile Metabolism

Carbon Source	Energy Source: Light	Energy Source: Chemical
CO_2	Photoautotrophs	Chemoautotrophs
Organic Compounds	Photoheterotrophs	Chemoheterotrophs

PNSB - Different light & Carbon sources

6

Objective

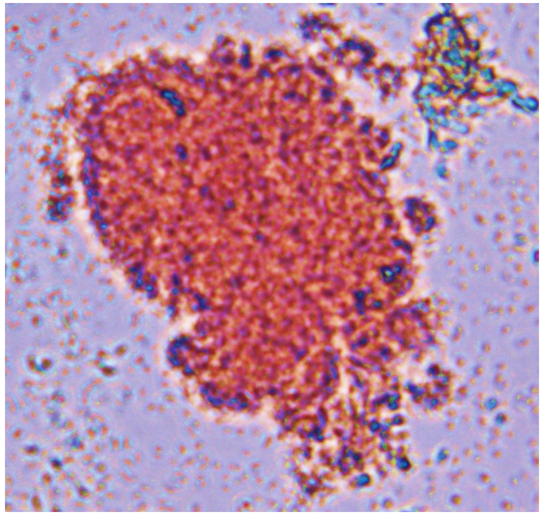
- The main objective of this study is to treat fuel synthesis wastewater using PNSB biofilm technology and recover various resources including carotenoids, bacteriochlorophyll, single cell protein and PHAs.

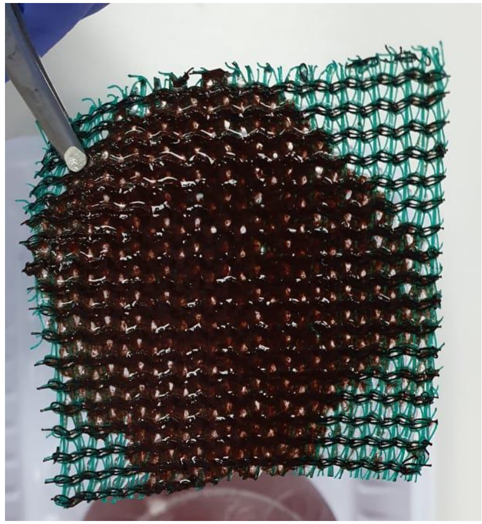
Motivation/Novelty

Proposed System (Economical)

Why?


Source: Google





Carbon substrate (wastewater)
- High carbon content.

PNSB strain
- Easy to enrich under non axenic conditions
- High potential for resource recovery.
- Does not require oxygen.
- Various C sources.

PNSB Biofilm
- Concentrated biomass.
- Easy harvesting.

Materials & Methods

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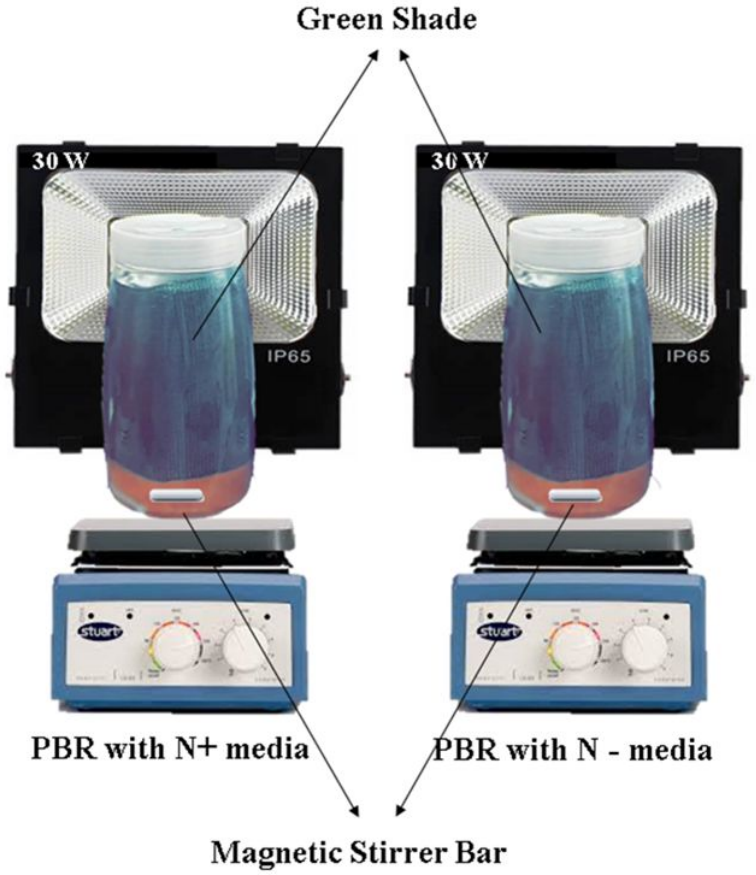
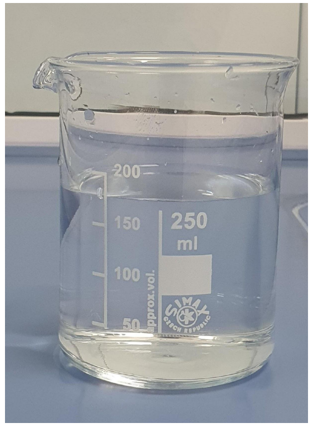
Biofilm photobioreactor, Culture condition, Microorganism and growth media



Biofilm photobioreactor used in this study

Mixed Culture of PNSB



Fuel Synthesis Wastewater

NH_4Cl (3.03 g/L)

KH_2PO_4 (3.03 g/L)

Na_2CO_3 (4.29 g/L)

TMS (10 mL /L)

VS (10 mL /L)

Composition of ATCC Trace Mineral and Vitamin Supplement

ATCC Trace Mineral Supplement	
Element	**Concentration (g/liter)**
Ethylenediamine-tetraacetate-di-Na-salt	0.5
Iron (II) sulfate heptahydrate	0.1
Boric acid	0.010
Cobalt (II) Nitrate hexahydrate	0.1
Manganese (II) sulfate hydrate	0.5
Zinc sulfate heptahydrate	0.1
Nickel (II) chloride hexahydrate	0.020
Sodium molybdate dihydrate	0.010
Copper (II) sulfate pentahydrate	0.010
Magnesium sulfate heptahydrate	3.0
Sodium chloride	1.0
Calcium chloride anhydrous	0.1
Aluminum potassium sulfate dodecahydrate	0.010
Sodium tungstate dihydrate	0.010
Sodium selenite	0.001
ATCC Vitamin Supplement	
Element	**Concentration (mg/liter)**
Folic acid	2
Pyridoxine hydrochloride	10
Riboflavin	5
Biotin	2
Thiamine hydrochloride	5
Nicotinic acid	5
Calcium Pantothenate	5
Vitamin B12	0.1
p-Aminobenzoic acid	5
Thioctic acid	5
Monopotassium phosphate	900

Materials & Methods (Determination of PNSB biomass and analysis of Effluent)

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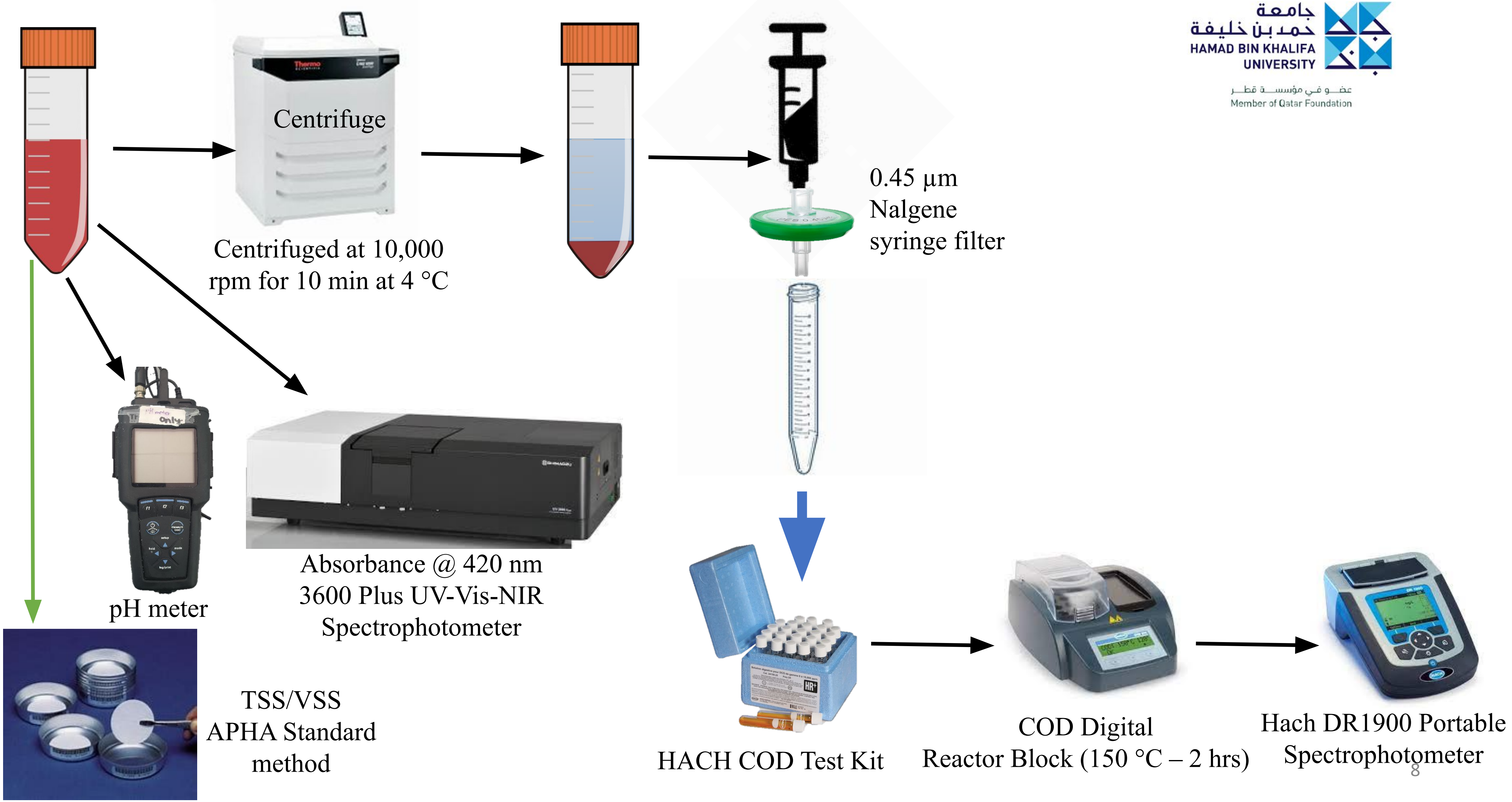

Materials & Methods (Determination of Hydrophobicity)

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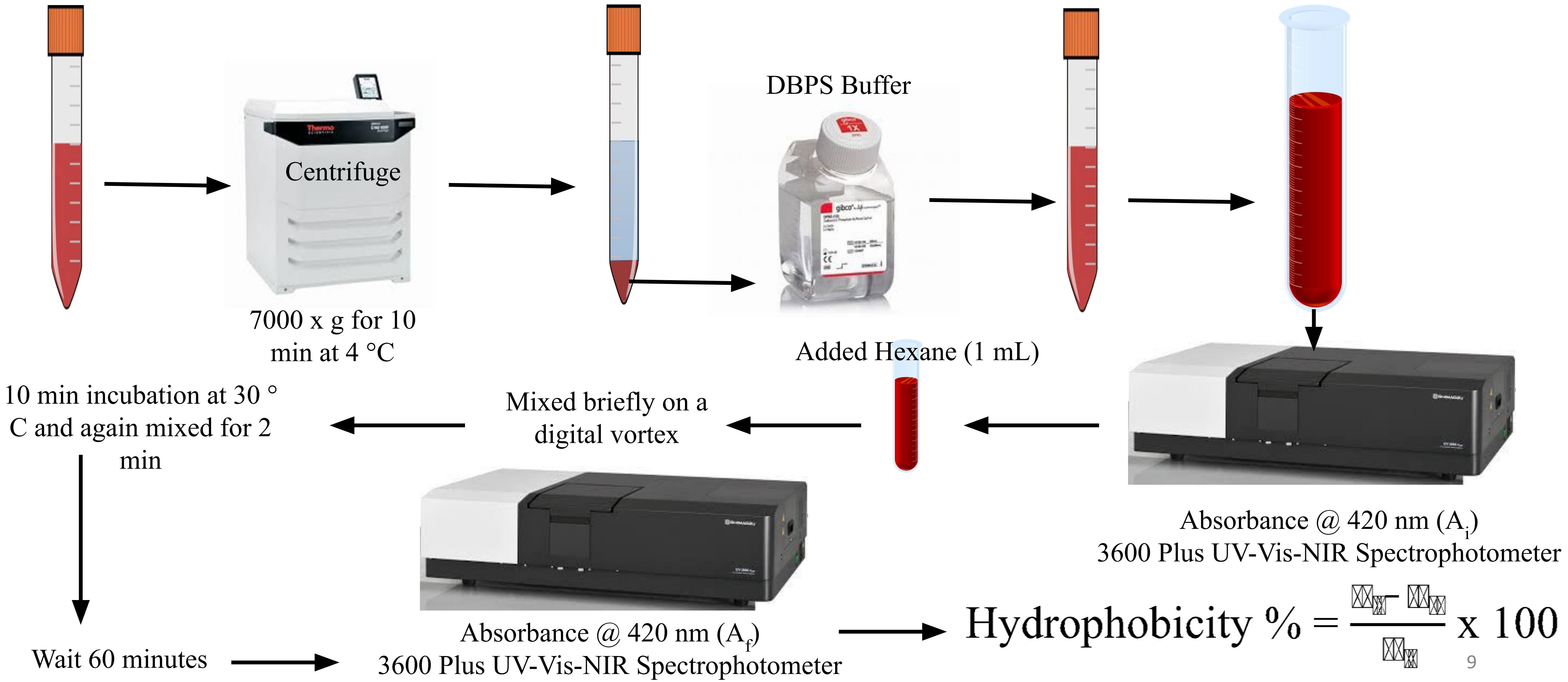

Materials & Methods (EPS Extraction and determination of EPS - PS and EPS – PN)

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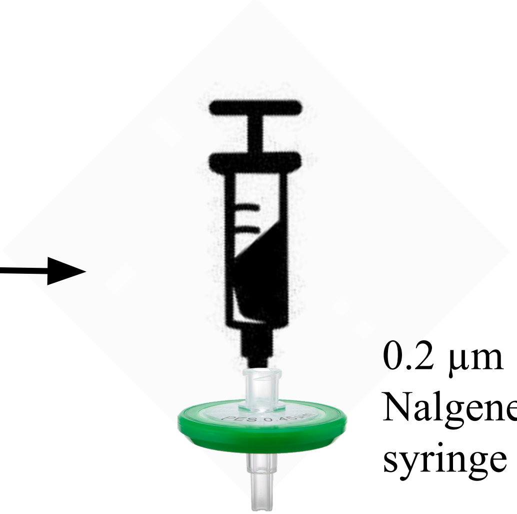
Extraction of EPS → **0.06 % Formaldehyde (mixing at 4 ° C) & heating at 80 °C for 10 minutes** →

Centrifuged at 20,000 x g for 20 min at 4 °C

→



0.2 µm Nalgene syringe filter

→ **EPS - Polysaccharides** → **Phenol-sulfuric colorimetric method**

→ **EPS - Proteins** → **Lowry protein assay method**

Materials & Methods (Extraction & determination of Crts & BChls)

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Extraction of Crts → **Acetone as Solvent** → 3600 Plus UV-Vis-NIR Spectrophotometer

Absorbance @ 480 nm

→ Carotenoids production $(mg\ L^{-1}) = \frac{[illegible]\ 10}{2500\ [illegible]_2}$

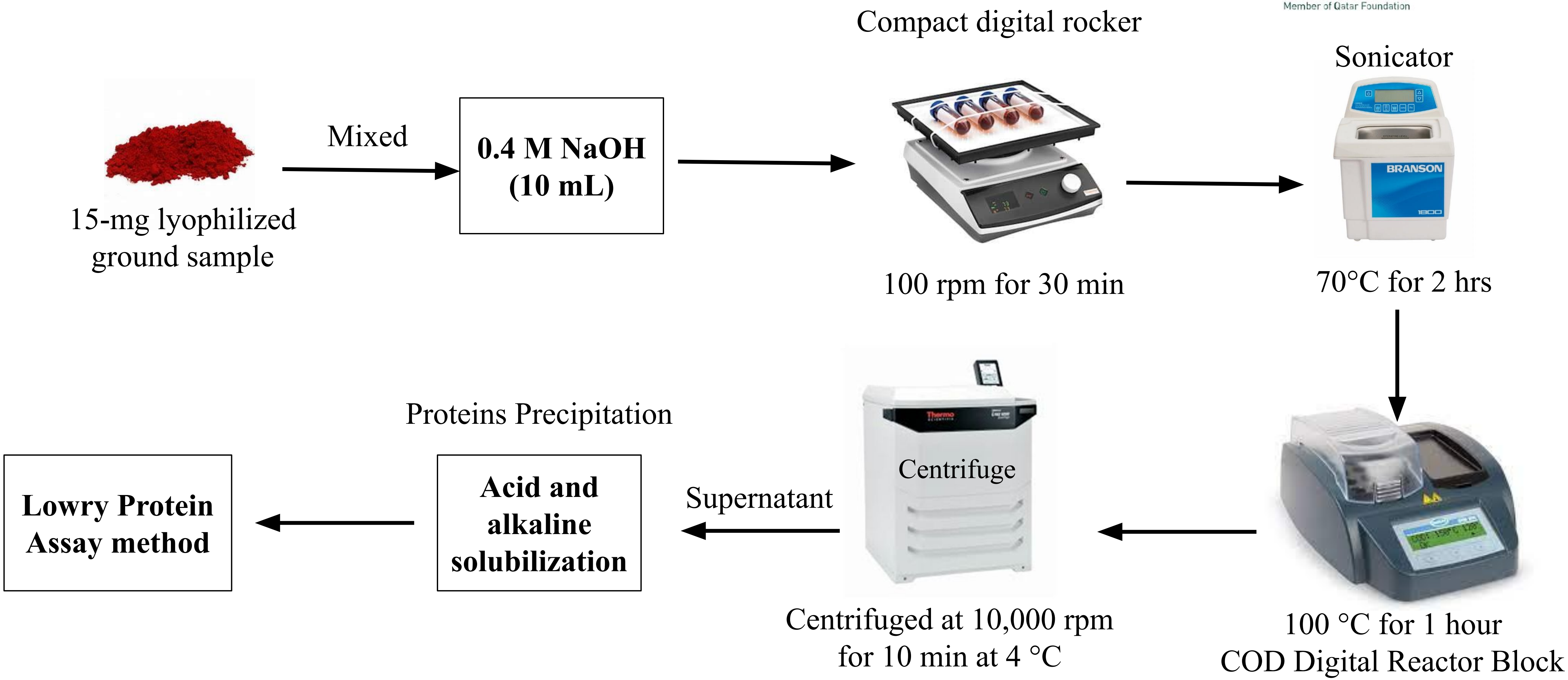
Extraction of BChls → **Acetone/Methanol 7:2 v/v as Solvent** → 3600 Plus UV-Vis-NIR Spectrophotometer

Absorbance @ 771 nm

→ BChl content $(mg\ L^{-1}) = \frac{[illegible]771\ [illegible]_1\ [illegible]}{760\ [illegible]_2}$

Materials & Methods

Extraction & determination of cellular protein



Materials & Methods

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Extraction & determination of PHAs

Extraction of PHB → Sodium hypochlorite dispersion method → 3600 Plus UV-Vis-NIR Spectrophotometer

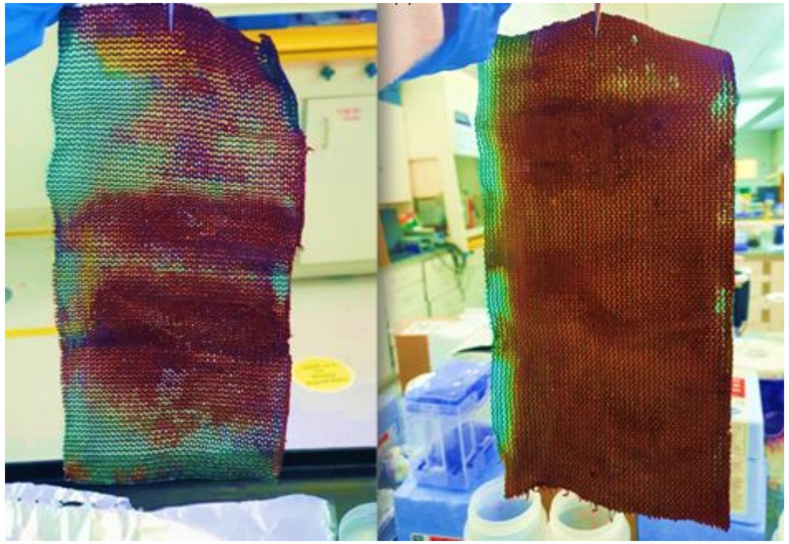
3600 Plus UV-Vis-NIR Spectrophotometer

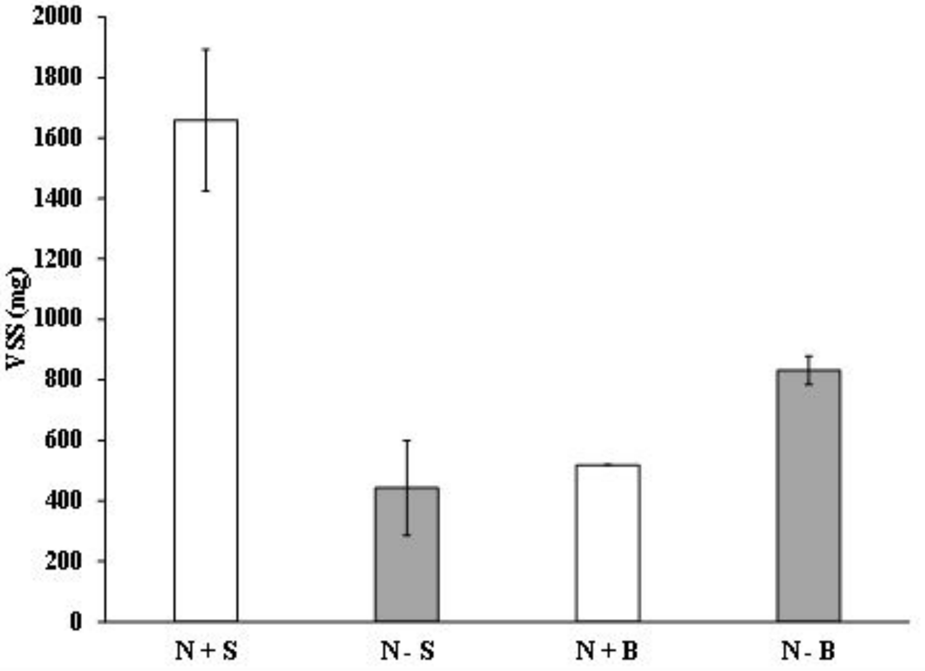
Absorbance @ 235 nm

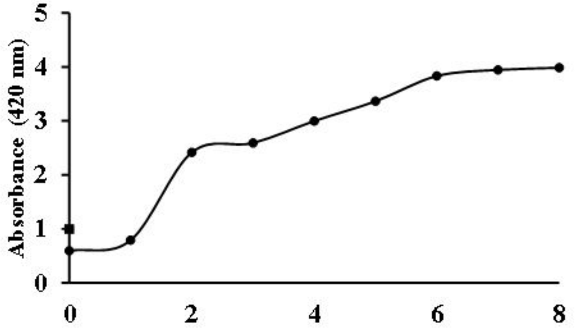
Results & Discussion

Effect of nitrogen on PNSB biofilm formation and wastewater treatment

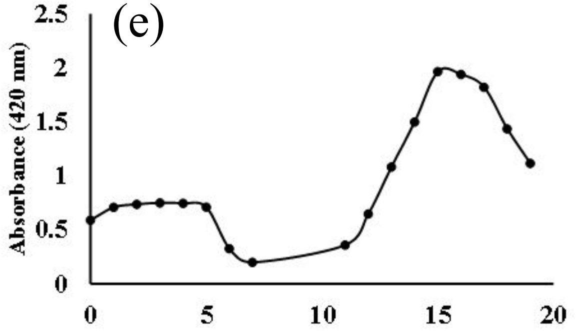
(a) (b) (c) (d)







(e)

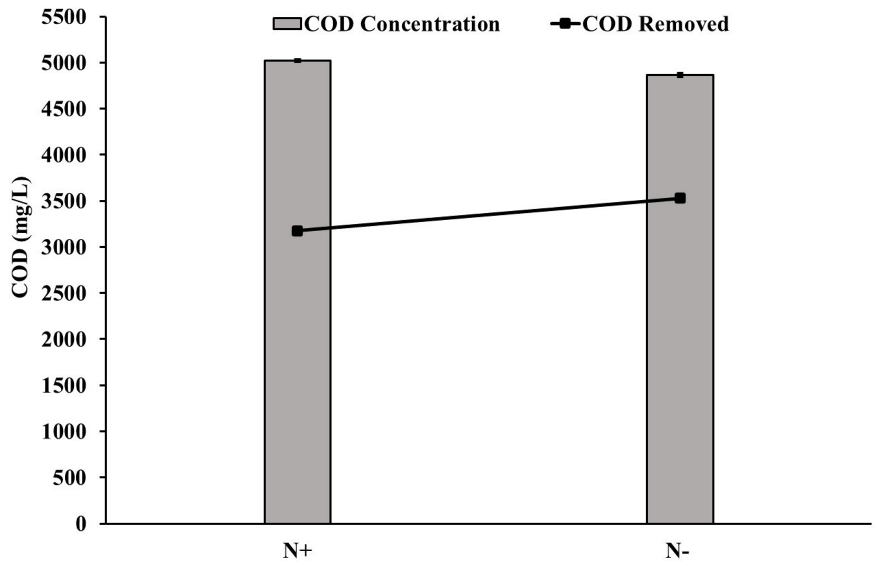


Biofilm formation in (a) N+ condition and (b) N- condition. (c) VSS in N+ and N- condition. Suspended culture growth measured by absorbance in (d) N+ condition and (e) N- condition

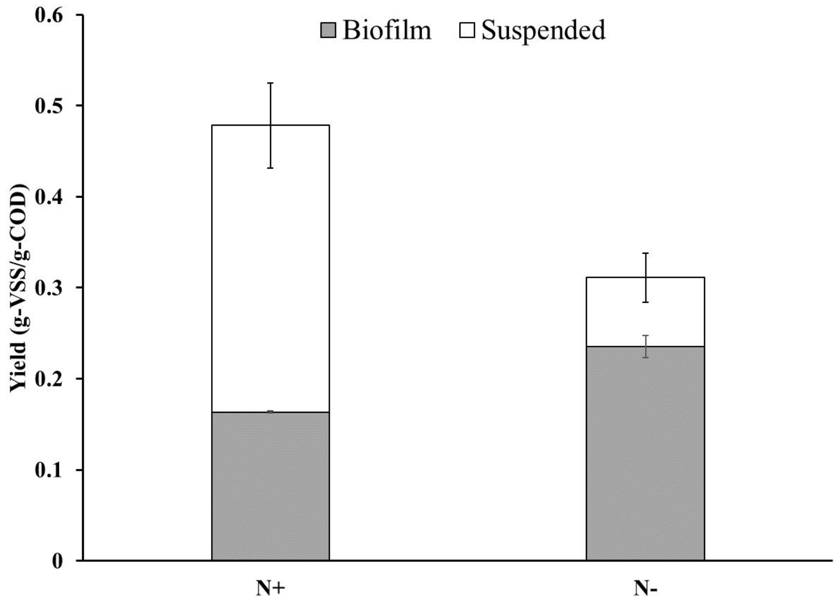
Results & Discussion

Effect of nitrogen on PNSB biofilm formation and wastewater treatment

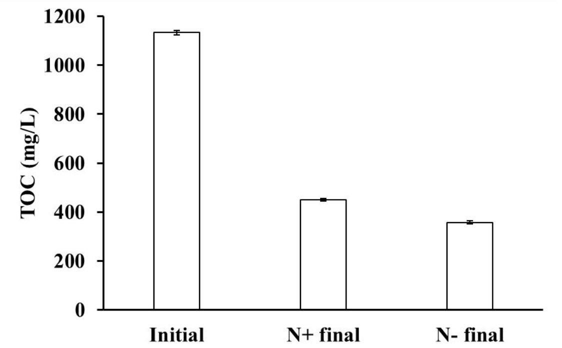
(a)



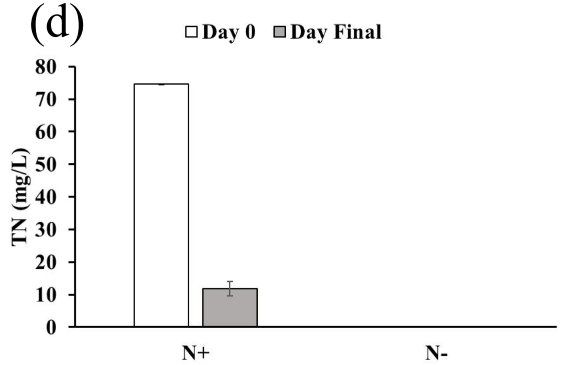
(b)



(c)



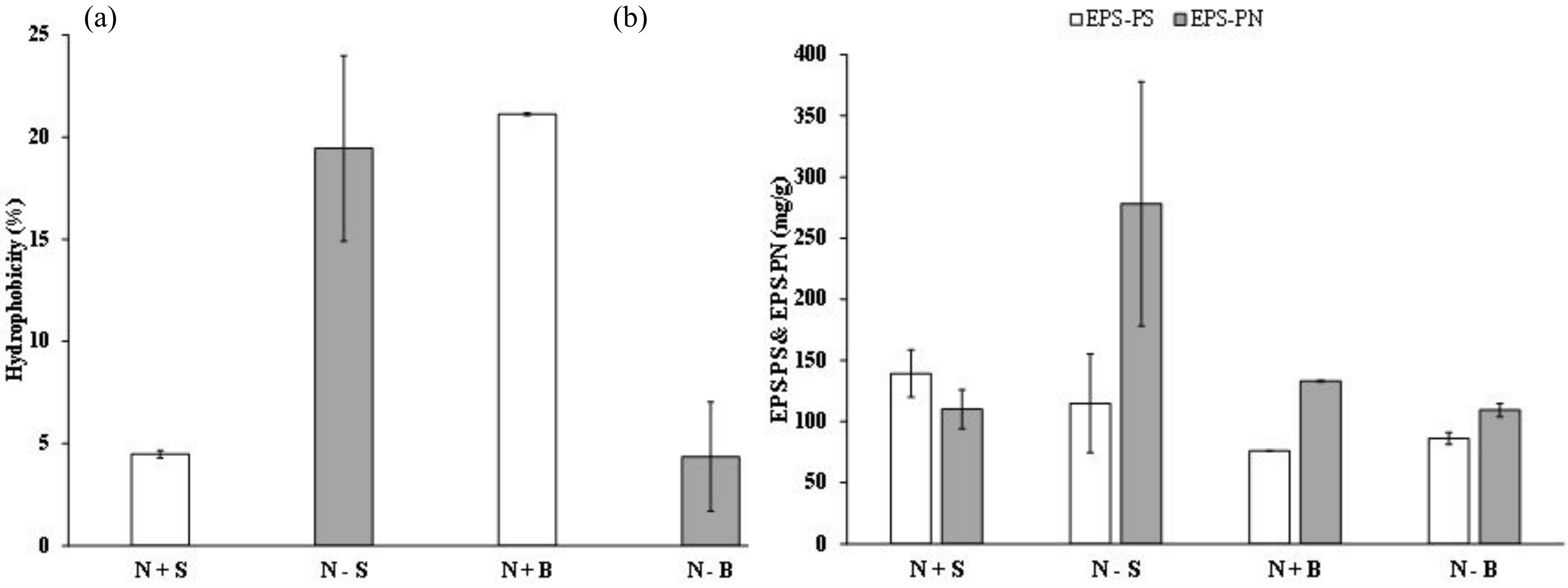
(d)



(a) COD concentration and COD removed (b) Biomass yield (c) Total organic carbon (d) total nitrogen at day 0 and day final in N^+ and N^- condition

Results & Discussion

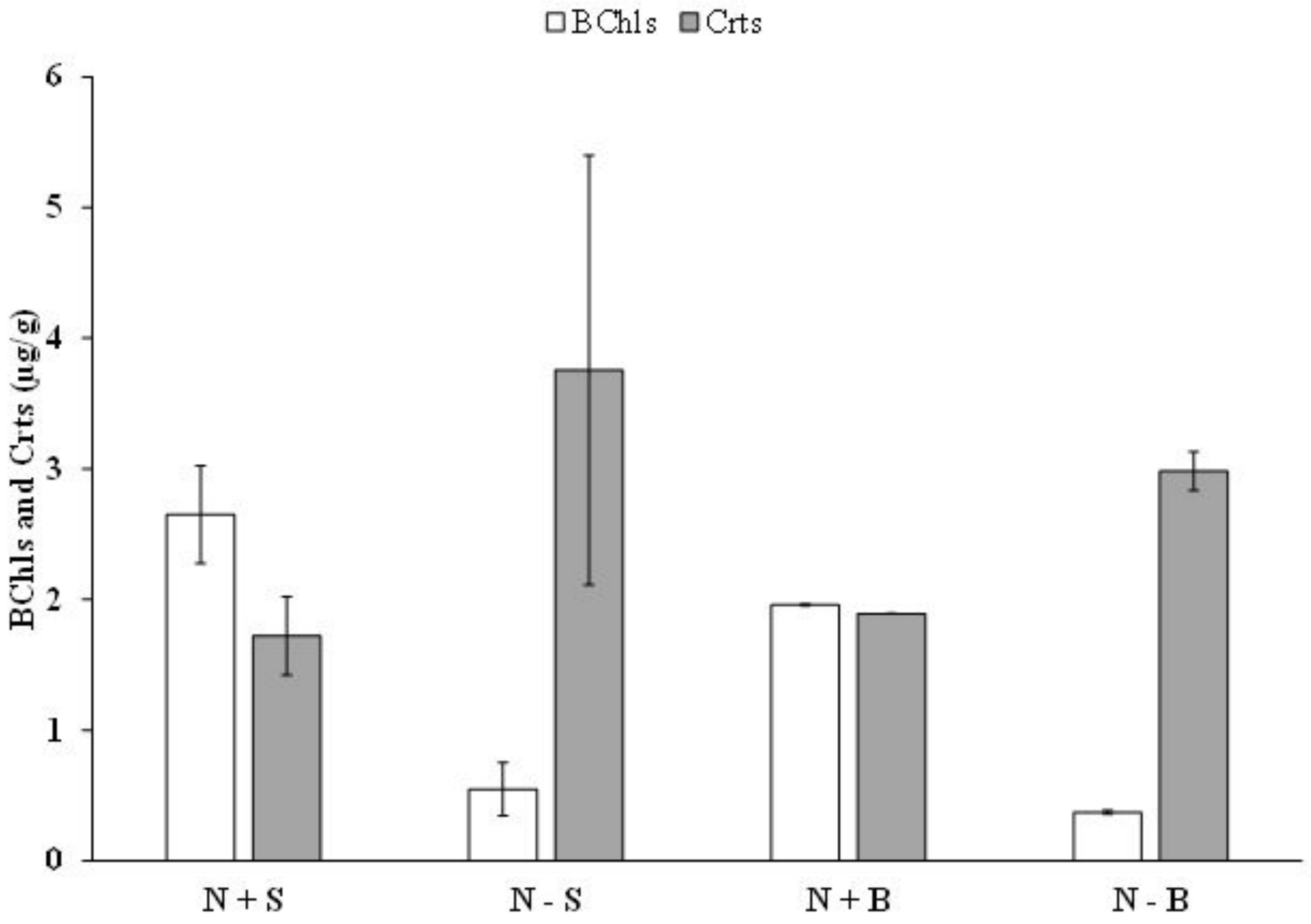
Effect of nitrogen on hydrophobicity, EPS-PS and EPS-PN



(a) Hydrophobicity (b) EPS-PS and EPS-PN of suspended and biofilm culture of N^{+} and N^{-} condition

Results & Discussion

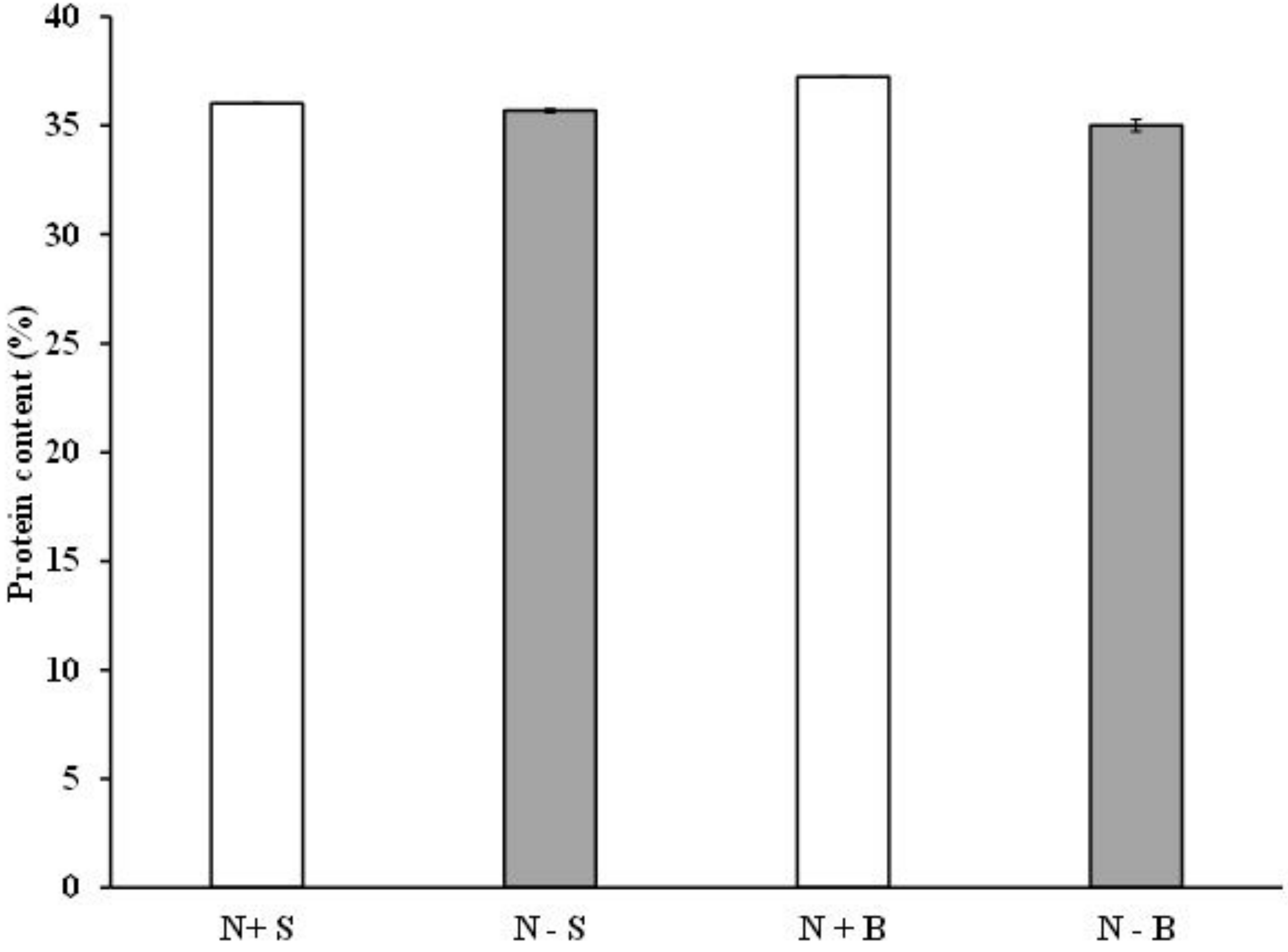
Effect of nitrogen on bacteriochlorophyll and carotenoids production



BChls and Crts production in N^+ and N^- condition

Results & Discussion

Effect of nitrogen on cellular protein production

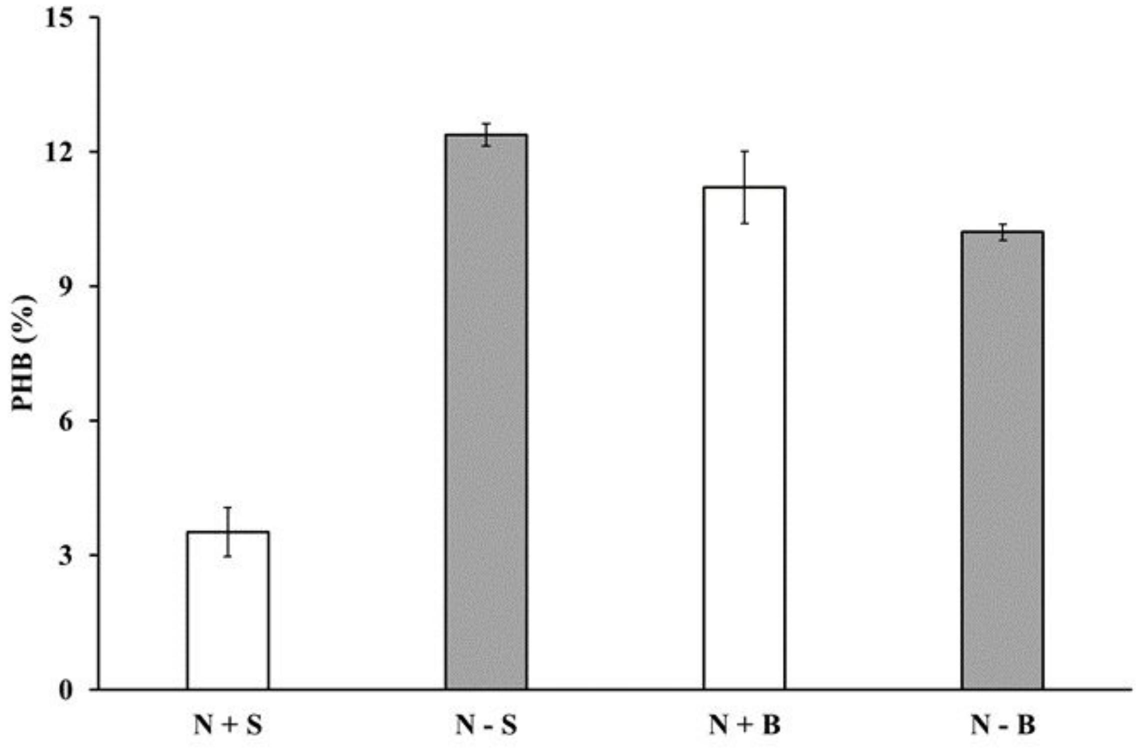


Protein content in N^{+} and N^{-} condition

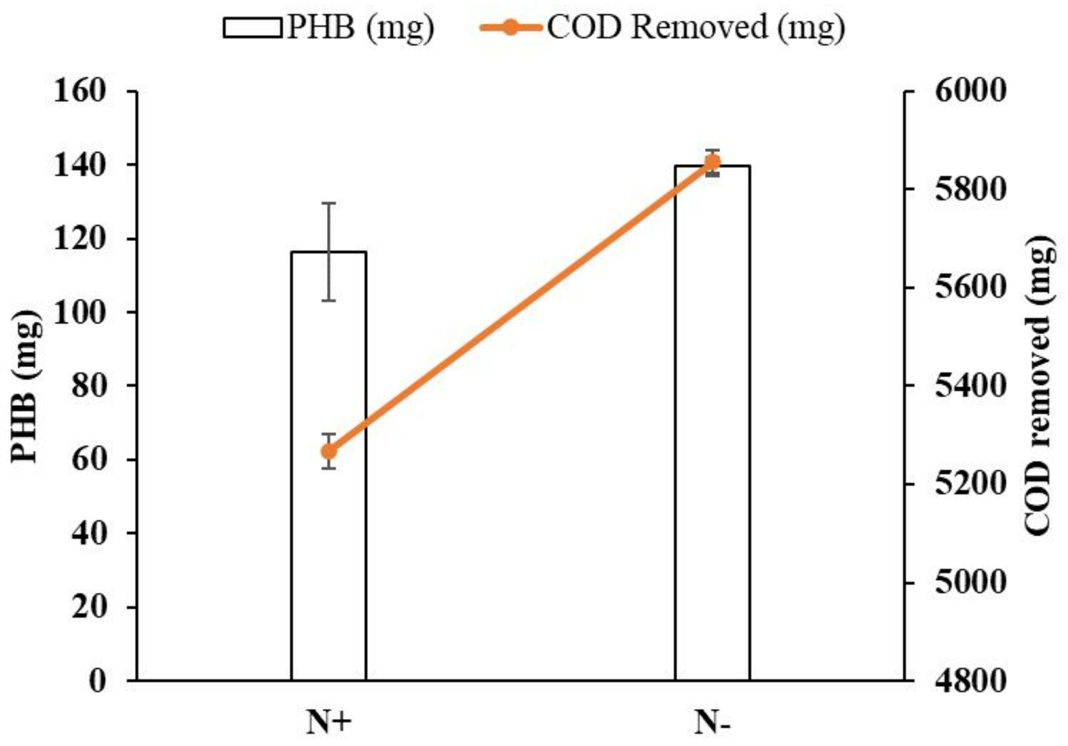
Results & Discussion

Effect of nitrogen on PHAs Production

(a)



(b)



(a) PHA content (b) COD removed vs PHB produced in N^+ and N^- condition

Conclusion

- PNSB enriched photobioreactor with N- media promoted increased biofilm formation relative to the proportion of suspended biomass.
- Despite the lack of nitrogen in one condition, the protein content in biomass from both conditions and culture was the same, indicating that nitrogen addition is not required to provide high protein content in biomass for SCP use.
- The average PHB content in N- condition was also higher than that of N+ conditions.
- The use of biofilm growth to help separation has no detrimental effects on the final product, although it does necessitate more research into suitable reactor designs.
- N deficient biofilm-based PNSB photobioreactors have the potential for effective COD removal and recovery of various high value-added resources including carotenoids and single cell protein, and possibly with optimization PHB.

ACKNOWLEDGEMENTS

- Qatar National Research Fund (MME01-0910-190029) and Qatar Shell Research and Technology Centre for their financial and technical support, respectively.

THANK YOU FOR YOUR ATTENTION

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