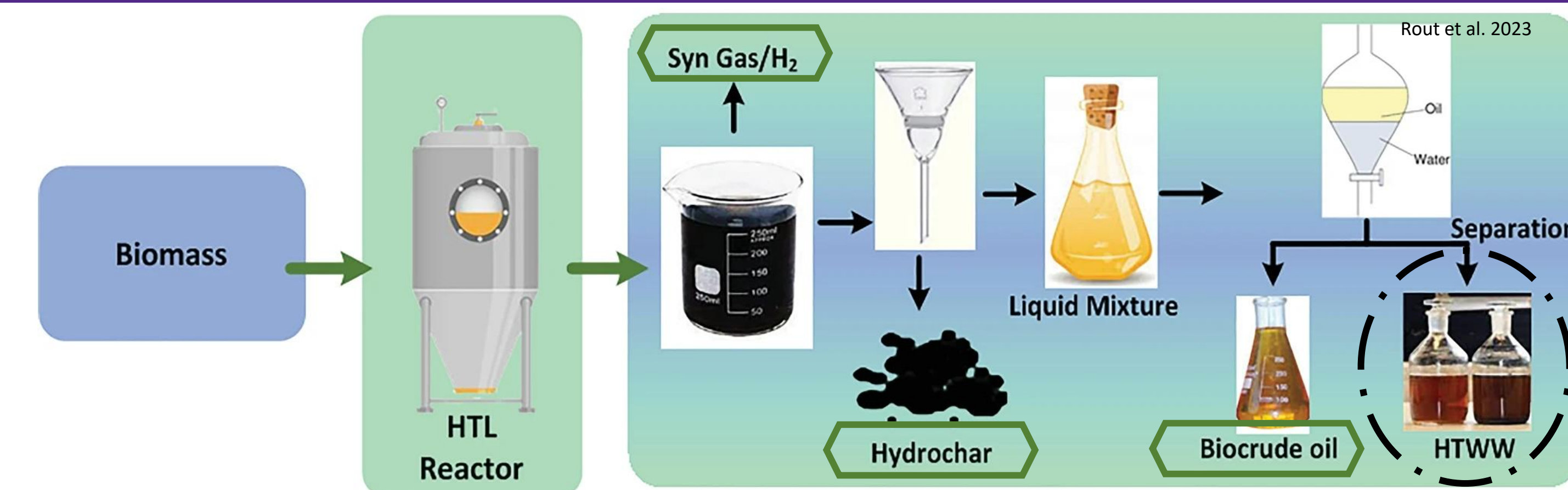
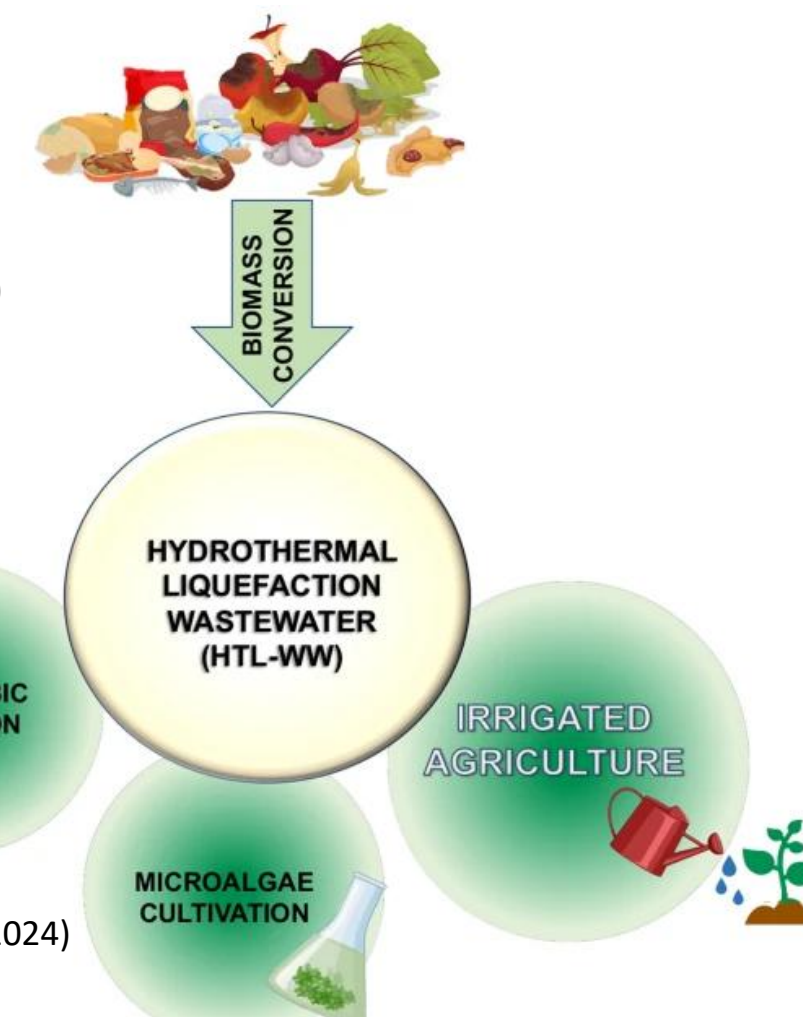
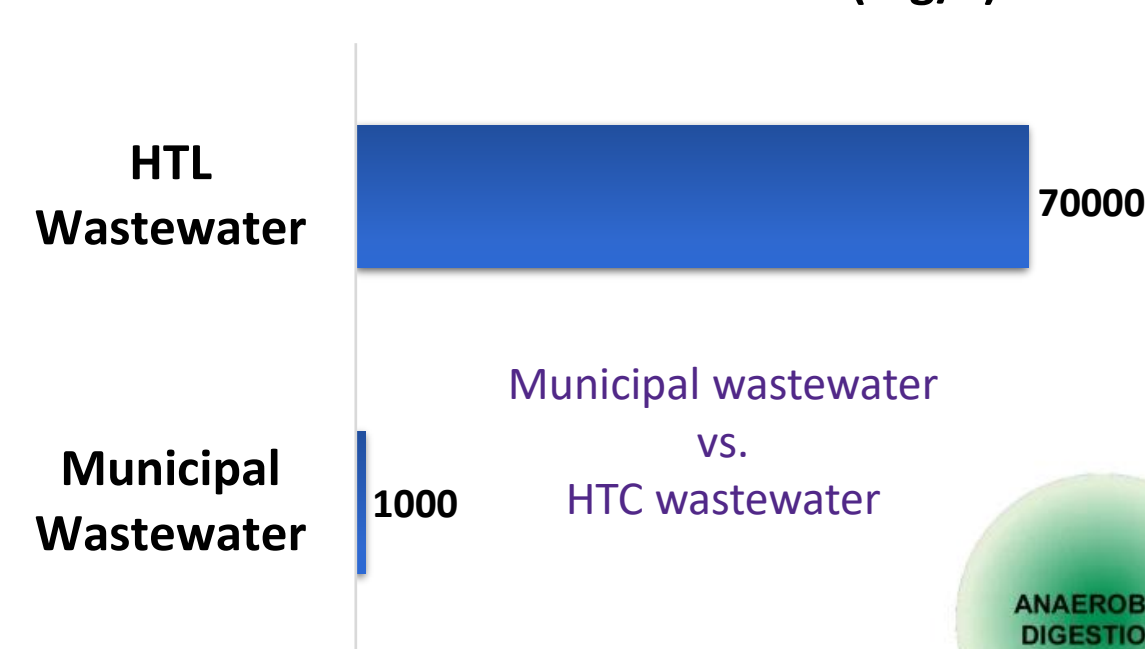




Background



Pollutant Concentration - COD (mg/L)



Conventional HTCWW management

- Composting /anaerobic digestion (AD)
- Irrigated agriculture

Biomass source

- Kitchen food waste
- Lignocellulosic biomass such as corn stover and hemp biomass
- Sewage sludge
- Municipal solid waste (mixture of plastic, paper, food, crushed glass and metal)

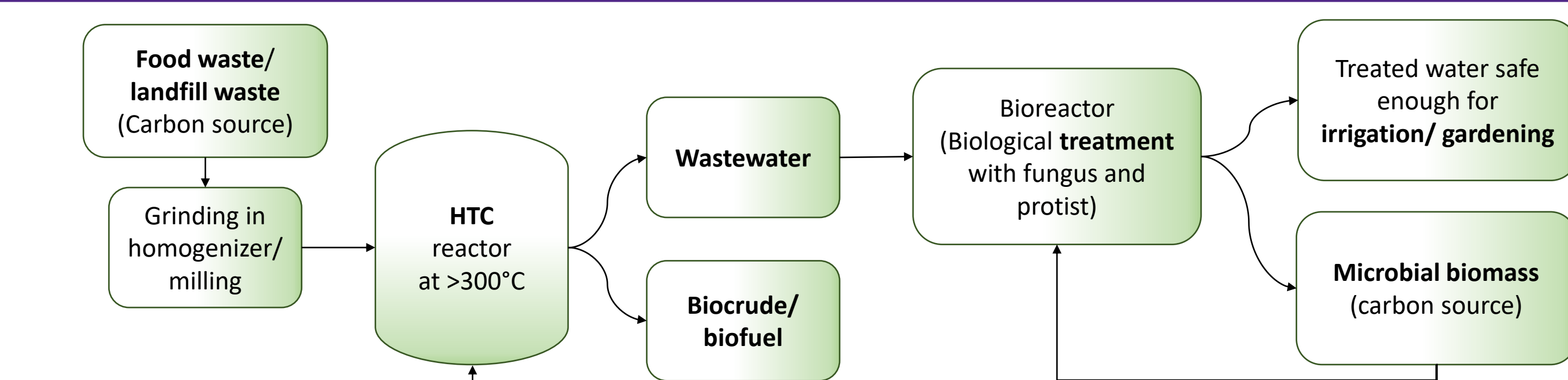
Problem encountered

- Inhibition to microbial degradation during AD and harmful to direct application to irrigation due to the presence of **metal impurities, toxic chemicals** such as furfural, organic acids, phenolic compounds and N-heterocyclic compounds

- Upcycling /valorization of HTCWW



Objectives



Upcycling of HTCWW

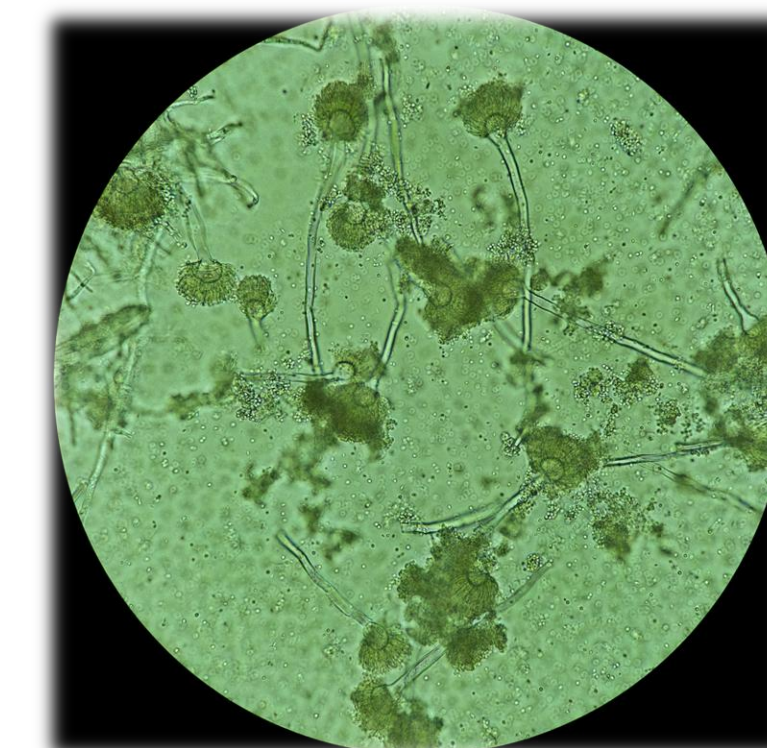
- Treatment of HTCWW with marine protist and fungal consortium to yield **bio-granules**.
- Activated sludge production and preservation.
- Recirculation of bio-granules to the HTC reactor as a **carbon source**.
- Lower chemical oxidation demand (COD) in treated wastewater to make it **environmentally safe**.

Relevance to Kansas

As Kansas embarks on new **thermal treatment technologies** for wastewater, this research supports the state's progress by addressing the **growing challenges of wastewater** from both households and biofuel industries. By harnessing Kansas's rich agricultural potential, this study encourages **expanding industries beyond biodiesel into other promising biofuels such as biocrude, syngas, and biochar**. Together, these efforts create a sustainable path to **protect water resources** and strengthen Kansas's renewable energy future.

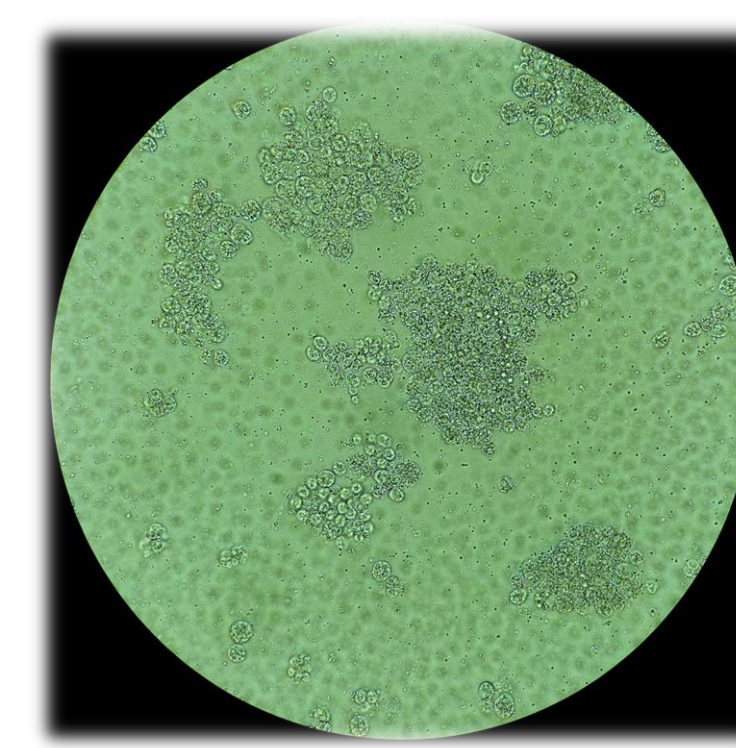
Methods

Microorganisms used



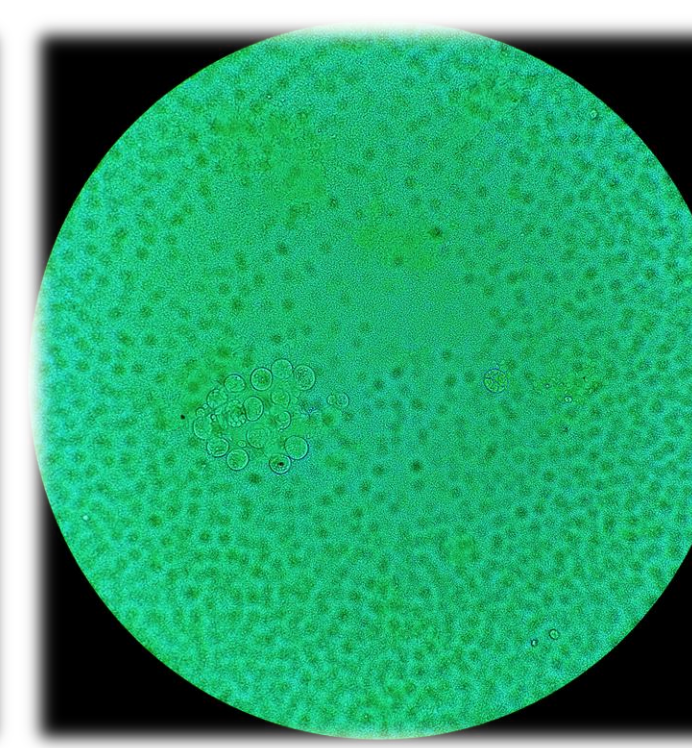
Aspergillus terreus (I)

Fungus



Schizochytrium sp. (II)

Marine protists



Thraustochytrium striatum (III)

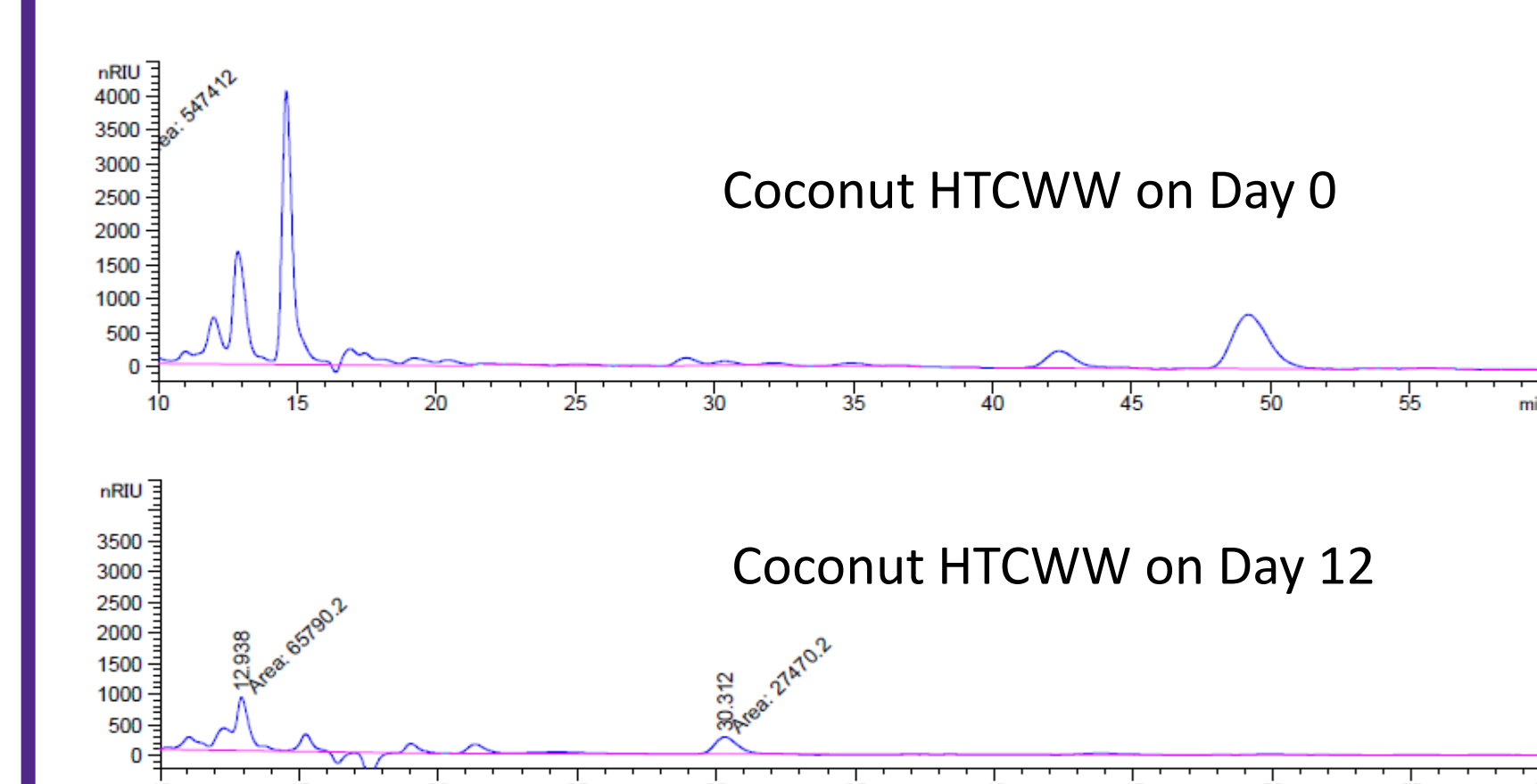
Dark fermentation (in flask)

- HTCWW was prepared by Dr. Kumar's group at Old Dominion University at 350°C for 5 min
- Biomass with respective **dilution factors** was used to prepare individual HTCWW:
 - Coconut shell @ 10X
 - Pasta @ 6X
 - Walnut shell @ 8X
- 3 fungal-protist combination** was used: (I+II), (I+III) and (I+II+III)
- Artificial Seawater** (ASW) was prepared mimicking the salt concentrations in seawater to ensure the thriving of marine protists
- Supplemental **nutrient salts** were determined and optimized in the ASW media using MTT assay in well plate
- Influence of **glucose supplementation** was observed
- A full cycle of **12 days fermentation** was conducted at 25°C with an agitation speed of 150 rpm to maximize the cell mass based on the depletion of carbon content in the media
- An intermediate **COD testing** was done at the regular interval of 3 days starting from the zeroth day
- Organic salts, furfural and phenolics concentrations** in the media were observed via HPLC at the regular interval of 3 days starting from the zeroth day
- Spent media except **activated biomass** was replenished with fresh media for the next cycle.

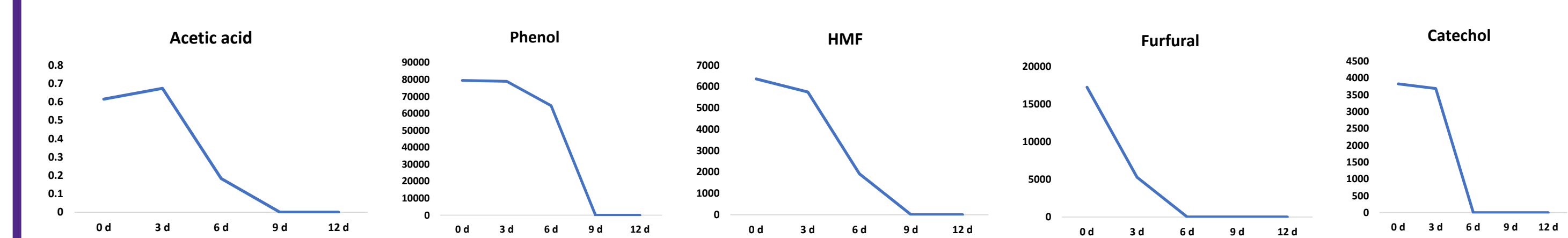


Results

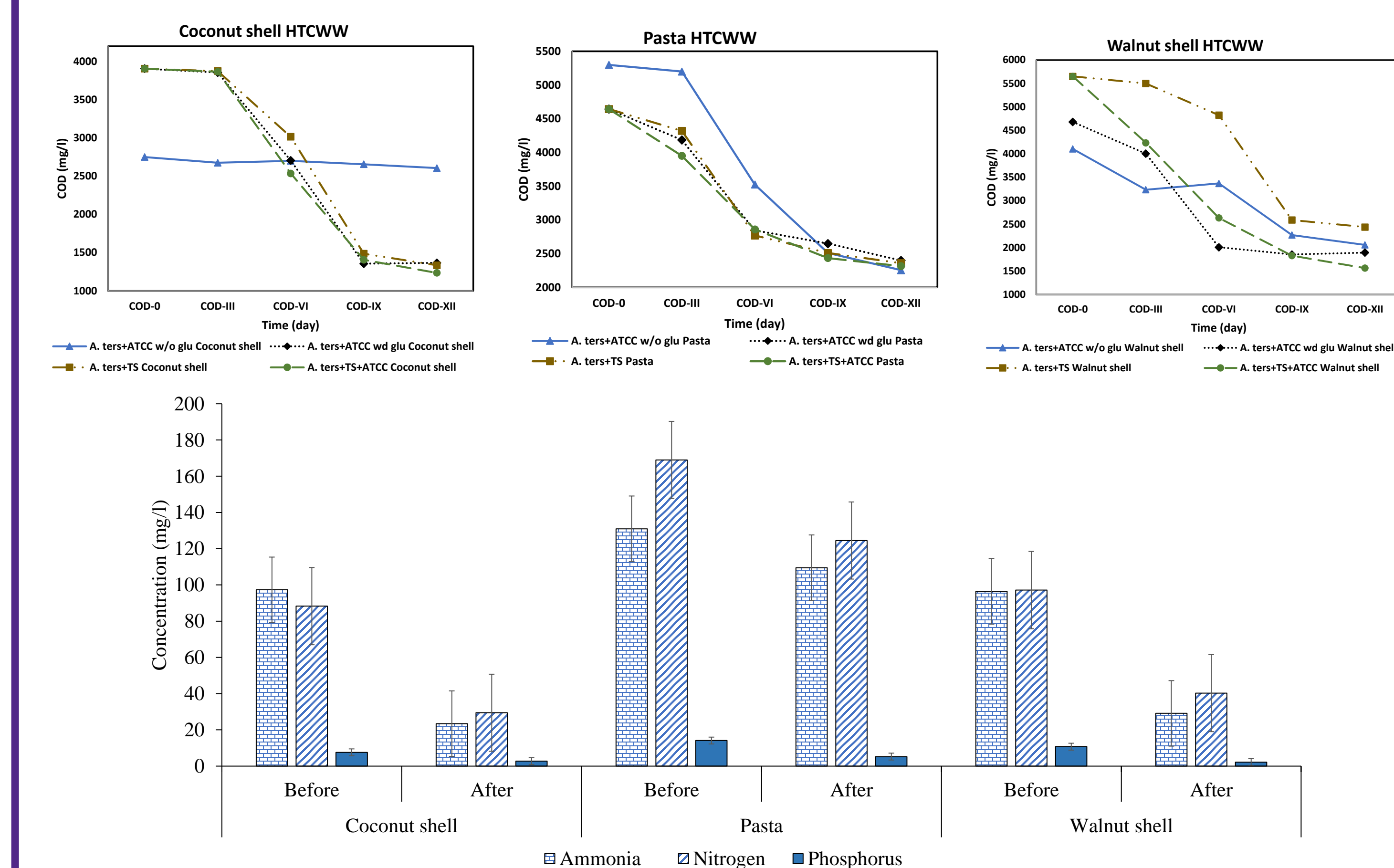
HPLC analysis on HTCWW



- Organic acids except glycolic acid were consumed initially along with furfural and phenols.
- Majority of the peaks including unidentified ones were reduced.



- Coconut HTCWW contained the highest levels of phenolic compounds and furfural, followed by walnut and pasta, which likely inhibited microbial growth. Therefore, dilution factors of **10x, 8x, and 6x** were applied, respectively.
- Nitrogen, phosphorus and iron** had significantly different effects on the microbial growth while glucose supplementation was observed to support initial growth though it did not contribute to overall COD removal.
- Activated biomass's** achieved similar results in 6 days of second cycle compared to the 9th day's progress of first cycle. Accumulated biomass at the end of fermentation were **recirculated back to the HTC reactor** for more biofuel production.
- Coconut shell HTCWW achieved the highest COD removal (**79%**), followed by walnut (**71%**) and pasta (**57%**).
- Majority of **phosphorus and nitrogen content** in wastewater from coconut shell and walnut shell were **consumed**, unlike in pasta HTCWW



Conclusions and Future Work

- CONCLUSION:** The **combination of both protists with fungi** was observed to remove almost **80% of original COD** in wastewater and was effective as activated biomass in the next cycle, compared to the other two combinations.
- FUTURE PROSPECTS:** Scaling up the process using a reactor with **fed-batch or continuous-feed operation** and **protein extraction/ ammonia removal** before treatment could increase efficiency.

Acknowledgements

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