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Continuous gas processing without bubbles using thin liquid film bioreactors containing biocomposite biocatalysts

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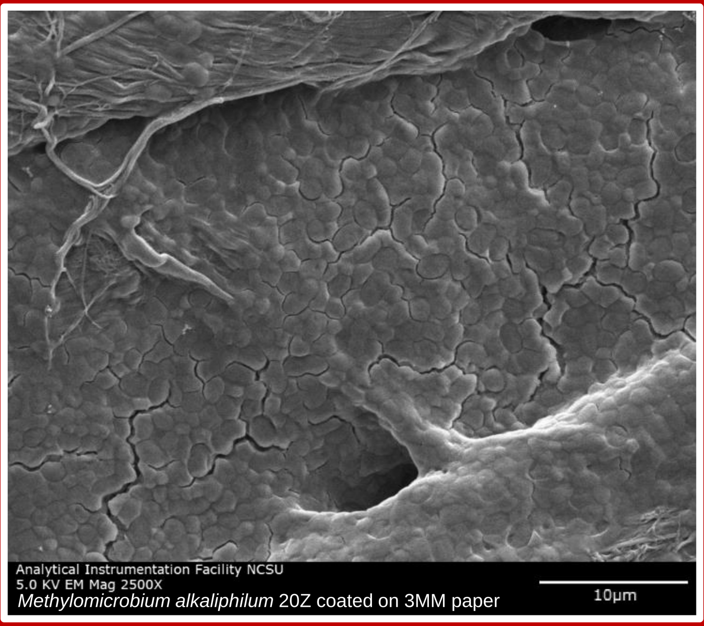

NC STATE UNIVERSITY

CONTINUOUS GAS PROCESSING *WITHOUT BUBBLES* USING THIN LIQUID FILM BIOREACTORS CONTAINING BIOCOMPOSITE BIOCATALYSTS

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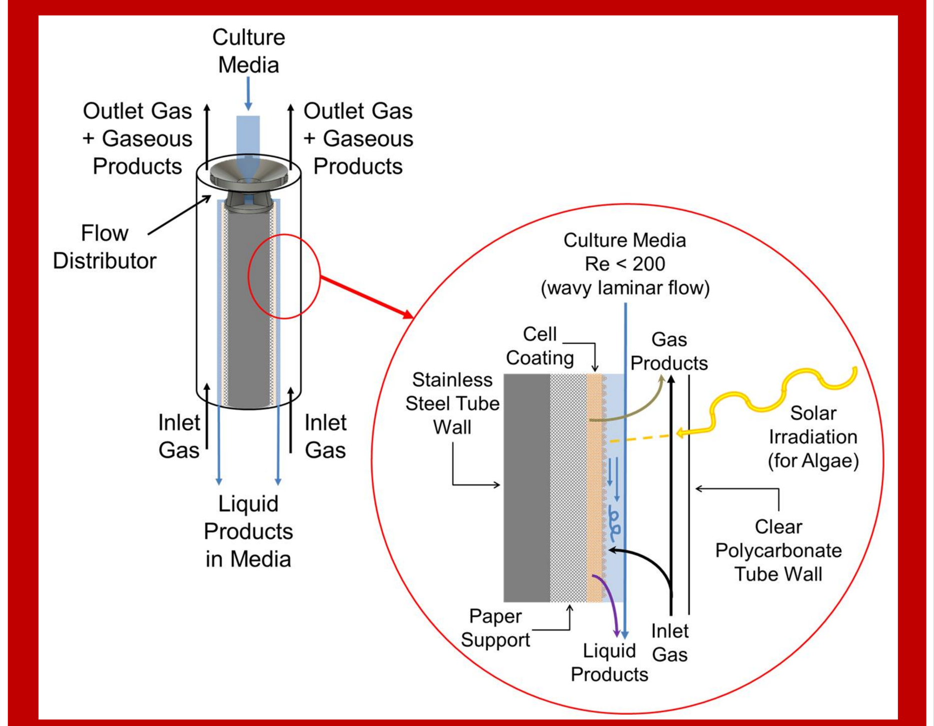
0.9 m x 0.04 m^2 prototype Falling Film Bioreactor (FFBR): gas vol. 235 ml, film vol. ~6 ml, total recirculating liquid vol. 65 ml

Abstract

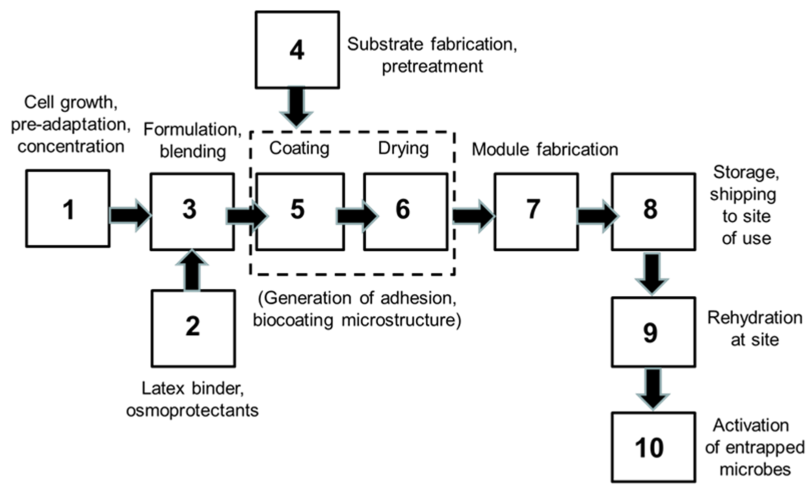
Energy efficient continuous microbial gas processing without bubbles is possible with a uniform ~300 μm thick falling film, plug flow bioreactor (FFBR). Power input is minimized by laminar wavy flow (Re <200) over a rough hydrophilic paper surface coated with concentrated living, non-growing microbes — a biocomposite biocatalyst. Falling films can increase mass transfer rates >100 fold compared to bubble aeration, decrease reactor volume, decrease gas-liquid (G-L) mass transfer energy input, decrease water use, and increase secreted product concentration. Paper roughness enhances gas-liquid-microbe (G-L-S) mass transfer, which is simulated using a 2D finite element method (FEM) CFD model (COMSOL). The paper functions as a separation device; the cells are retained and secreted products continuously removed. Microbes concentrated in paper biocomposites take up gas at higher rates than suspended cells at low mass transfer power input. This also increases specific activity for some photosynthetic organisms. We are investigating FFBR biocomposite biocatalyst proof-of-concept using a 0.9 m, 0.04 m^2 prototype cylindrical paper bioreactor. Model systems we investigate include *Clostridium ljungdahlii* OTA1 (absorbing CO from syn-gas), *Methylomicrobium alkaliphilum* 20Z (absorbing CH_4 in air), and cyanobacteria or microalgae (absorbing CO_2). Critical are coating microstructure, wet microbe adhesion (latex binders, engineering the surface of the microbes), surviving osmotic shock in coating formulation/controlled drying (lyoprotectants), and desiccation tolerance to prolonged dry storage. Spatially correlated Raman micro spectroscopy and hyperspectral imaging have been developed as a method to monitor the distribution of residual water surrounding and within the cells. The distribution of vitrified residual water may contribute to desiccation resistance. This technology may also be applied to a spinning disk bioreactor (SDBR), that enhance mass transfer by reducing liquid film thickness to <100 μm with wave induced turbulent flow using centrifugal force (1000 x g).

Thin falling film bioreactor



Non-growing living cells in a paper biocomposite biocatalyst enable continuous gas processing, continuous product separation

Goal: Dry-stabilized modules for continuous gas processing



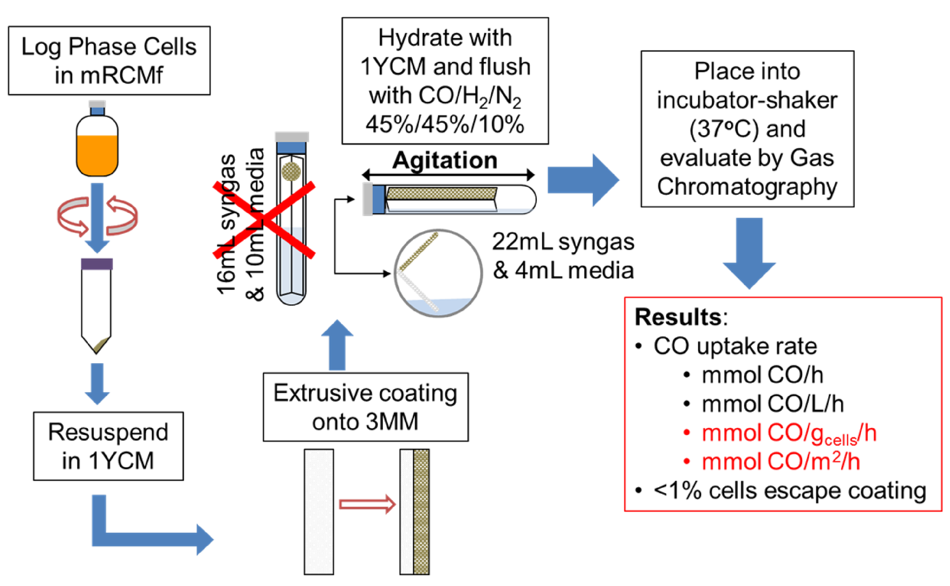
Cellular stresses that occur during biocomposite fabrication, module storage (steps 3, 5, 6, 8)[1,2]

- osmotic stress
- desiccation stress
- oxidative damage

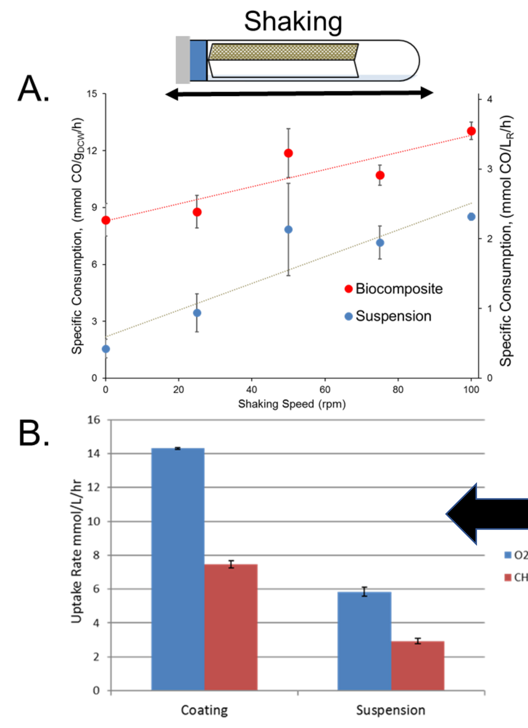
Tube Reactivity Screen → FFBR, G-L-S Mass Transfer, Experiments, CFD Modeling

Model nitrogen-limited microbes used for FFBR biocomposite biocatalyst design

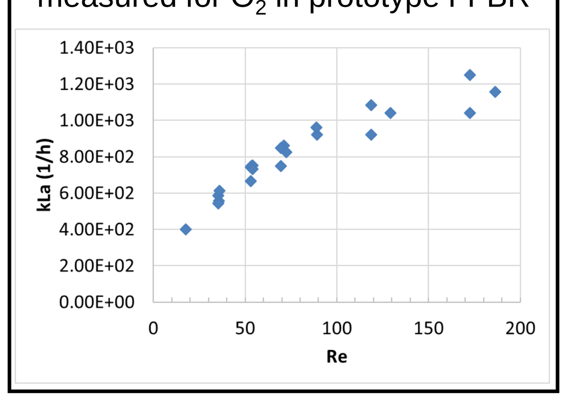
- *Clostridium ljungdahlii* OTA1 absorbing CO + H_2 → organic acids[5]
- *Methylomicrobium alkaliphilum* 20Z absorbing CH_4 + O_2 → organic acids (MA)
- Microalgae, cyanobacteria absorbing CO_2 → O_2 + secreted products[4]



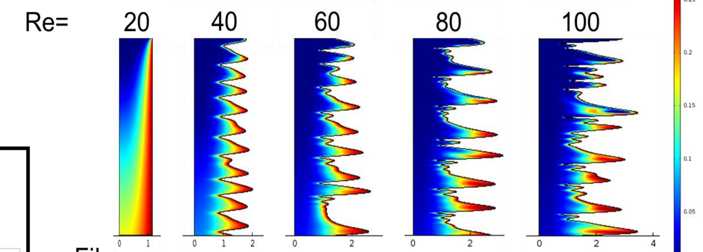
Horizontal tube gas-absorbing reactivity screen, *C. ljungdahlii* OTA1 in paper biocomposite above liquid, tube 85% gas, 15% liquid, slow horizontal shaking to minimize mass transfer power input[5]



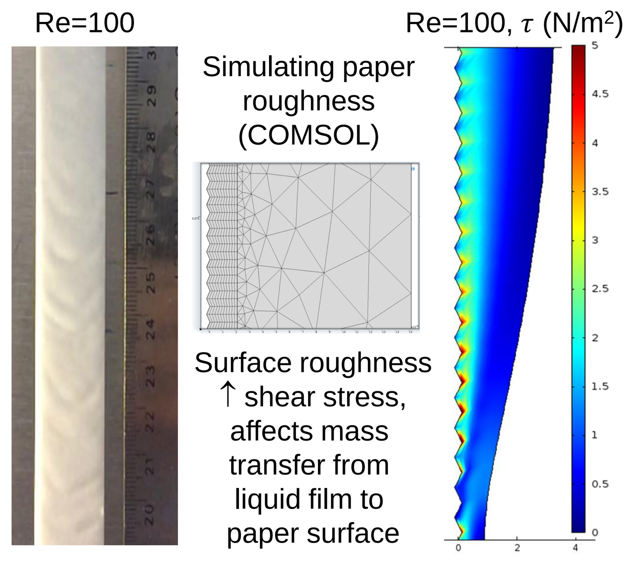
Gas uptake by biocomposites compared to suspended cells
A. *C. ljungdahlii* OTA1[5]
B. *M. alkaliphilum* 20Z



Simulating falling film mass transfer, 2D FEM 5x faster than FVM (COMSOL)[9]



Falling film thickness, velocity, dissolved O_2 experimentally measured

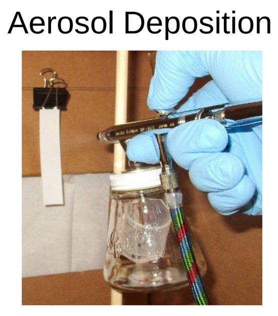


Biocomposite Biocatalyst Fabrication on Paper

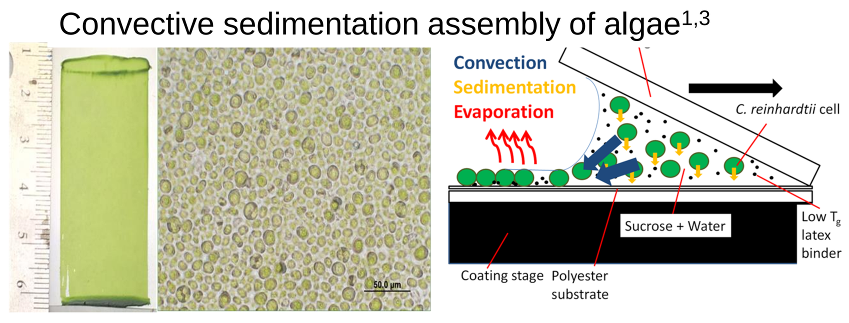
Formulation/drying critical for cell layer adhesion, viability following rehydration

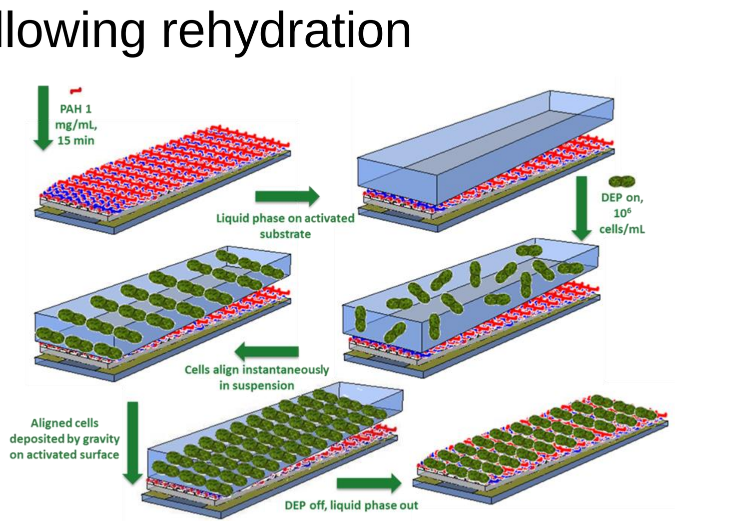
- Mayer rod drawn, extrusion deposition, microbial paper[7]
- Aerosol deposition (air brush)
- Convective sedimentation assembly (CSA)
- Dielectrophoresis (DEP) on polyelectrolyte layers

Aerosol Deposition

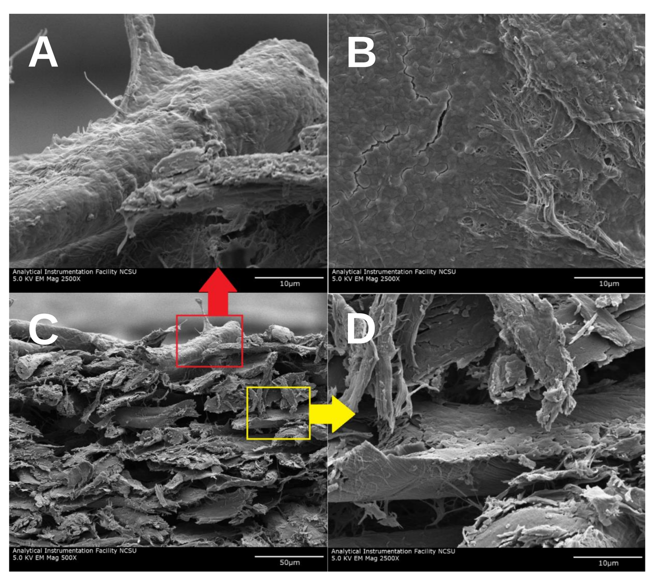


Convective sedimentation assembly of algae[1,3]





Dielectrophoresis (DEP) of cyanobacteria on a polyelectrolyte-coated surface[6]



SEM images of aerosol-coated 3MM paper with *M. alkaliphilum* 20Z: A cells on paper fiber, B top view, C cross-section, D region without cells

Conclusions

- Falling films (Re<200) on rough paper generate wavy laminar flow; high G-L mass transfer
- Paper roughness enhances liquid film-cell coating (S) mass transfer; G-L-S mass transfer simulated using a 2D FEM rough surface CFD model
- Biocomposites of non-growing microbes consume more gas than growing cells at low power input
- FFBR predictions from *C. ljungdahlii* OTA1 syn-gas uptake results predict significant reduction in power input; reduced water use, reduced reactor volume (PI) compared to STR
- Prototype plug flow FFBRs developed as proof-of-concept for continuous processing of CH_4 in air using *M. alkaliphilum* 20Z
- FFBR paper support retains biocatalysts, continuously separates secreted products in falling liquid phase (reaction + separation)
- Methods developed for coating paper with concentrated non-growing microorganisms (>10^{12} CFU/m^2) stabilized dry
- Desiccation-resistance is achieved by cellular engineering, drying under lyoprotective conditions; residual water monitored by confocal Raman microspectroscopy OH:CH_2 peak intensity
- Key aspects: microbe wet adhesion to papers, wavy laminar flow, minimal outgrowth (growth limitation), desiccation tolerance (lyoprotection)
- Surface expression of cellulose binding modules (CBMs) underway to generate binding of *M. alkaliphilum* 20Z to paper
- *M. alkaliphilum* 20Z being engineered to secrete muconic acid (MA) – biological route to adipic acid

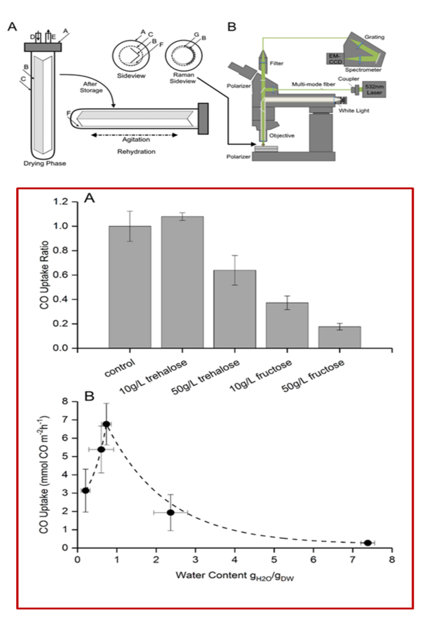
Collaborators

btec Biomanufacturing Training and Education Center; M DEARBORN; Newcastle University; NREL

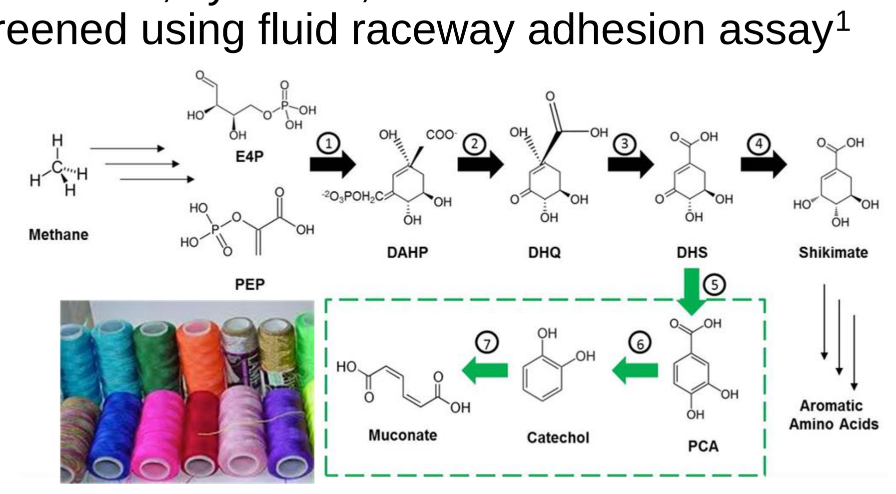
Cellular Engineering for Adhesion to Paper, Lyopreservation by Water Vitrification, Product Secretion (Muconic Acid)

Carbohydrate Binding Module (CBMs) overexpression in *M. alkaliphilum*

- Expression targets: OmpA tethering, native S-layer protein tethering
- CBM donor organisms: *C. thermocellum, M. capsulatus, T. reesei*
- CBM domain protein origins: cellulosomal scaffolding protein, endoglucanase/exoglucanase, xylanase, chitinase
- Adhesion to paper screened using fluid raceway adhesion assay[1]



Optimal residual water for lyo-preservation of *C. ljungdahlii* OTA 1 activity after rehydration monitored by confocal Raman micro spectroscopy[8]



Pathway engineering in *M. alkaliphilum* 20Z for overproduction/secretion of muconic acid (MA) from methane

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Predicted Impact of FFBR - Process Intensification

Process Intensification (PI), reduced water, power predicted using modular continuous FFBR for syn-gas processing

Reactor Type	Reactor Volume (m^3)	Water needs (m^3/day)	Power needs (kW)
Continuous Stirred Tank	59	29	59
Biocomposite Falling Film 20 X 1m^3 modules	20 66% Smaller	4.8 83% Less Water	2.2 96% Less Power

CSTR data for processing 1,000 m^3 CO/day, D = 0.02 h^{-1} calculated from Richter *et al., Energies*, 2013

Recent publications by our group

1. Flickinger, et al., *J. Coatings Technol. Res.* **14:**791-808 (2017)
2. Cortez, et al., *Biochem. Eng. J.* **121**, 25-37 (2017)
3. Jenkins, et al., *Materials* **6**, 1803-1825 (2013)
4. Bernal, et al., *Biotechnol. Bioeng*. **111:**1993-2008 (2014)
5. Schulte, et al., *Biotechnol. Bioeng.* **113:**1913-1923 (2016)
6. Bernal, et al., *Langmuir* (2017) doi 10.1021/acs.langmuir.7b00335
7. Bernal, et al., *BioResource* **12:** 4013-4030 (2017)
8. Schulte & Solocinski, et al., *PLoS One* (2017)10.1371/journal.pone.0180806
9. Schulte, et al., (2017) (submitted)