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A high gas fraction, reduced power, syngas bioprocessing method demonstrated with a *Clostridium ljungdahlii* OTA1 paper biocomposite

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Abstract

We propose a novel approach to continuous bioprocessing of gases. A miniaturized coated-paper high gas fraction biocomposite absorber has been developed using slowly shaken horizontal anaerobic tubes with concentrated *Clostridium ljungdahlii* OTA1 absorbing syngas as a model system. These gas absorbers demonstrate elevated CO mass transfer with low power input, reduced liquid requirements, elevated substrate consumption, and increased product secretion compared to shaken suspended cells. We concentrate OTA1 in a cell paste which was coated by extrusion onto chromatography paper. Cell adhesion was by adsorption to the cellulose fibers; visualized by SEM. The *C. ljungdahlii* OTA1 coated paper mounted above the liquid level absorbs CO and H₂ from a model syngas producing acetate with minimal ethanol. At 100 rpm shaking speed (7.7 W m⁻³) the optimal cell loading is 6.5 g_{DCW} m⁻² to maintain high CO absorbing reactivity without the cells coming off of the paper into the liquid phase. Reducing the medium volume from 10 mL to 4 mL (15% of tube volume) did not decrease CO reactivity. The reduced liquid volume increased secreted product concentration by 80%. The specific CO consumption by paper biocomposites was higher at all shaking frequencies <100 rpm than suspended cells under identical incubation conditions. At 25 rpm the biocomposite outperforms suspended cells for CO absorption by 2.5 fold, with a power reduction of 97% over the power input at 100 rpm. The estimated minimum apparent k_{La} for these biocomposite gas-absorbers is ~100 h⁻¹, a *10 to 10⁴ less power input* than other syngas fermentation systems at similar k_{La} . Specific consumption rates in a biocomposite were measured as ~14 mmol g_{DCW}⁻¹ h⁻¹. This work intensified CO absorption and reactivity by 14 fold to 94 mmol CO m⁻² h⁻¹ over previous *C. ljungdahlii* OTA1 work by our group. Specific acetate production rates were 23 mM h⁻¹ or 46 mmol m⁻² h⁻¹. The specific rates and apparent k_{La} were shown to scale linearly with biocomposite coating area. These proof of concept results will be used to engineer a continuous biocomposite gas absorber bioreactor.

Introduction

Gas-to-liquid (GTL) biocatalysis is attractive for production of renewable fuels and chemicals because of substrate flexibility, product specificity, and mild reaction conditions (Buchholz, 2012). Genetic engineering can modify a platform organism to produce different products of interest from the same substrate (Drzyzga et al., 2015). However, GTL biocatalysis has not made significant penetration into the marketplace because it is limited by the energy cost for dispersion of low solubility gases into aqueous media, slow volumetric substrate consumption, slow specific production rates, batch processing, and low product titers (Abubackar et al., 2011; Bengelsdorf et al., 2013; Drzyzga et al., 2015; Wilkins and Atiyeh, 2011).

These limitations are particularly evident in stirred tank bioreactors. Suspension bioreactors consume a substantial amount of energy ($1,000\text{--}10,000\text{ W m}^{-3}$) to contact low solubility gases with suspended cells. The power required to disperse large volumes of gas into a large volume liquid phase increases operating costs. In the absence of cell recycle, the shear forces at high energy input limits the cell density and therefore gas consumption/liquid production rates. Much of the research in syngas fermentation is with batch large volume liquid systems. However, batch systems require larger facilities and capital expenditures than continuous processes. Finally, because of the lower cell concentration, mass transfer resistance, and often toxicity of the secreted products to the host organism, specific production rates are low in GTL microbial processes compared to sugar fermentations which can be $>10\text{ g L}^{-1}\text{ h}^{-1}$ ($>200\text{ mM h}^{-1}$) (Bayrock and Ingledew, 2005). Additionally, high secreted product concentrations require fermentations in excess of 5 days (Phillips et al., 1993; Shen et al., 2014a).

The most interesting gases industrially for GTL biocatalysis are CO, CH₄, and CO₂. Our model system uses *Clostridium ljungdahlii* OTA1 to convert CO from syngas as its sole carbon source into ethanol and acetate (Koepke et al., 2011; Ragsdale and Pierce, 2008; Tanner et al., 1993). Mutant OTA1 was derived from ATCC 55383 and isolated for its desirable ethanol to acetate ratio and CO consumption (Tirado-Acevedo, 2010; Whitham et al., 2015). Wild type *C. ljungdahlii* has been shown to have a maximum specific uptake of $\sim 35\text{ mmol CO g}_{\text{DCW}}^{-1}\text{ h}^{-1}$ in liquid culture (Mohammadi et al., 2014; Richter et al., 2013).

There have been a number of efforts to address the limitations in stirred tank syngas processes by investigating other gas-liquid contacting configurations. They are suspended systems such as bubble columns or airlift, or immobilized systems such as trickle bed, or microchannel monoliths. Hollow fiber reactors, which have been the subject of many recent investigations, can operate with cells in suspension (Davis, 2007) or immobilized on the shell side of the fibers (Shen et al., 2014a). As the solubility of CO is fixed by fermentation conditions, most researchers focus on improving the carbon monoxide mass transfer coefficient, k_La , as described in equation 1.

$$COTR = \frac{dn_{CO,l}}{V_L dt} = k_L a (C_{CO,l}^* - C_{CO,l}) \quad (1)$$

Where COTR, $n_{CO,l}$, $k_L a$, C^* , C_L , and V_L are the CO transfer rate ($\text{mol L}^{-1} \text{h}^{-1}$), moles of CO in the liquid phase, overall mass transfer coefficient (h^{-1}), the maximum CO solubility (mol L^{-1}), the concentration of CO in the liquid phase (mol L^{-1}), and the liquid volume, respectively. At large scale, reactor types that don't require mechanical agitation are preferred for good mass and heat transfer properties at lower power than stirred tanks (Shuler, 2002).

Both suspension and immobilized systems can face limitation at large scale in a continuous system. In order to achieve high production rates and product titers, suspended systems need a cell recycle step to maintain the catalyst in the system. However, at these very high cell densities, mass transfer of nutrients as well as gaseous substrates will limit the productivity per g_{DCW} (Richter et al., 2013). Many immobilized cell and trickle bed systems can demonstrate elevated mass transfer but rely on the organism's ability to form a thin biofilm with good adhesion and the volume of gas in the reactor is low due to the volume of the inert packing material (Schuler, 2002).

What is needed to process large volumes of low solubility gaseous carbon is to significantly reduced power for gas-liquid mass transfer, intensify volumetric gas uptake rates, enhance microbial specific productivity, decrease reactor size by using a continuous system, stabilize biocatalyst half-life, and minimize water/medium requirements for increased product concentration and more efficient product purification. This can be achieved by applying the concepts of process intensification (PI) from chemical reactor technology to bioprocesses to reduce process footprint and increase reactivity (Hessel et al., 2013; Jenck et al., 2004; Kumar and Nigam, 2012; Vaccaro et al., 2014; Wiles and Watts, 2012). Previous work on bioprocess intensification has been limited to colonization of macroporous monolith supports which demonstrated again the established limitations of reactor channeling, non-uniform flow and large pressure drops in a gas/liquid system (Akay et al., 2005).

In order to address the limitations described above and to increase the intensity of the reaction as well as the volume of gas in the reactor, we propose a novel reactor configuration using a biocomposite: cells immobilized on paper - an inexpensive flexible nonwoven porous matrix. Cells in a non-growth state are adsorbed onto cellulose fibers in a nonwoven paper support with controlled loading (coating) for uniform reactivity. This approach removes the cells from the bulk liquid phase and concentrates their syngas absorbing reactivity over a defined surface area with the gas phase separated from the microbes only by a thin liquid film (Gosse et al., 2012). The gas phase now dominates the volume of the reactor (high gas fraction). Using this approach in a falling film continuous bioreactor configuration, the nutrient medium flows down and through the biocomposite (surface and through paper pores space) and the gas flow is co-current. As a liquid film of controlled thickness separates the cells from the liquid phase, elevated mass transfer at low power input can be achieved. Falling film reactors demonstrate very high mass transfer rates in, for

example, nitrobenzene hydrogenation ($\sim 10,000 \text{ h}^{-1}$). Because of the small liquid phase volume, the interfacial area, a , can be as high as $20,000 \text{ m}^2 \text{ m}^{-3}$ of reactor volume in contrast to bubble columns and agitated tanks which do not exceed interfacial areas of $200 \text{ m}^2 \text{ m}^{-3}$ (Hessel et al., 2005). A large scale gas processing continuous reactor could be designed using many biocomposite modules in parallel.

Previous work on biocomposites addressed the limitations in batch processing and slow specific rates in *C. ljungdahlii* as well as other organisms for gas absorption/evolution and submerged liquid systems. By limiting nutrients so the entrapped cells are non-growing but metabolically active, the half-life of the biocatalyst can be extended so that the continuous system can operate over long periods without biocatalyst deactivation or generating waste biomass. Preliminary studies by our group on small surface area paper biocomposites in vertical tubes demonstrated sustained reactivity for over 300 hours for *C. ljungdahlii* OTA1 and 100s of hours for other photosynthetic microorganisms (Bernal et al., 2014; Gosse et al., 2012). A submerged hydrogen generating photoreactive biocomposite of *Rhodospseudomonas palustris* coated onto polyester strips demonstrated continuous reactivity at a constant rate without cell growth for over >4000 hours when periodically supplied with acetate (Gosse et al., 2010). Using biocomposites was shown to intensify specific consumption rate by ~ 5 to 10 fold for gas absorbing systems with *Synechococcus* (Bernal et al., 2014) and increase the effectiveness factor of liquid transformations by 20 fold in *Gluconobacter oxydans* (Fidaleo et al., 2006).

Here we report advances over our previous syngas biocomposite work (Gosse et al., 2012) by using horizontal tubes with increased coating surface area and reduced liquid volume. This new configuration demonstrates increased specific CO consumption, enhanced specific product production rate and concentration by liquid film reactor design, not engineering the cells. This horizontal tube batch system generates critical parameters for designing continuous falling film biocomposite gas absorbers such as power requirement, cell loading, surface area, coating structure, and minimum liquid volume.

Materials and Methods

Computational Methods

A simplifying assumption for solving for $k_L a$ is assuming $C_{\text{CO},l} \approx 0$. This is reasonable if there is a high concentration of active *C. ljungdahlii* OTA1. This assumption provides a minimal estimate for $k_L a$ referred to as the apparent overall mass transfer coefficient, $k_{L,a_{app}}$. Rearranging equation 1 yields:

$$k_{L,a_{app}} = \frac{COTR}{C_{CO,l}^*} \quad (2)$$

Estimation of the solubility of CO in the liquid phase was done using the initial partial pressure of CO and the Henry's constant corrected for temperature (Sander, 2015). In a batch system, C^* will change as a function of time, but this assumption gives a minimal estimate of $k_L a$.

Methods for estimating the power consumption of a shaking vessel have been described (Buchs et al., 2000a; Buchs et al., 2000b; Kato et al., 1995). Accurately determining power requirement for a small volume shaking vessel is challenging. As such, modifications were employed to make them applicable for our horizontal tube system, to estimate shaking power requirements (Buchs et al., 2000b). Buchs defines the Reynolds (Re) number according to:

$$Re = \frac{\rho n d^2}{\eta} \quad (3)$$

Where ρ , n , d , and η are the liquid density, shaking frequency, maximum inner shaken flask diameter, and dynamic viscosity, respectively. The Reynolds number was empirically correlated with a modified power number Ne' by:

$$Ne' = \frac{P}{\rho n^3 d^4 V_L^{1/3}} = 70 Re^{-1} + 25 Re^{-0.6} + 1.5 Re^{-0.2} \quad (4)$$

Where V_L is the liquid volume in the shaking system. Equation 4 can be rearranged to solve for power consumption:

$$\frac{P}{V} = Ne' \rho \frac{n^3 d^4}{V^{2/3}} \quad (5)$$

Where P and V are the power requirement and liquid volume, respectively. Solving equation 5 gives an estimate of the power requirements at different shaking frequencies. We have modified this relationship to estimate the power input for liquids agitated by shaking in horizontal tubes where Re is low. The interior surface area of a horizontal tube that a known liquid volume covers at rest was calculated as d and used for the power calculation defined as a circle of the same area.

Buchs cautions that when Re is less than 10^4 , the shaking may lead to the liquid being “out-of-phase” where portions of bulk liquid are motionless. That may lead to large errors in the calculated power consumption using equation 4. This type of motion may be occurring in our slowly shaking horizontal tube system especially at the very low liquid volume and shaking frequencies where $Re \sim 10^3$. However, those errors are always an overestimation of the power requirement (Buchs et al., 2000b). Consequently, using the calculated values as an estimate for our tube system will result in the highest values.

Bacterial Strain, Media, and Growth conditions

Clostridium ljungdahlii OTA1, a mutant of *C. ljungdahlii* that has shown oxygen tolerance and improved product ratio (kindly provided by Dr. Amy Grunden, NCSU) (Tirado-Acevedo, 2010) was used as a model syngas absorbing organism. The growth medium, modified clostridial medium with fructose (mRCMf), was prepared as previously described

(Cotter et al., 2009) excepting that the pH was adjusted to 6.3 and each 160 mL serum bottle received 93 mL of medium and the headspace was 45% CO/45% H₂/10% N₂. In order to limit outgrowth from the biocomposite, 1YCM, was prepared as previously described (Gosse et al., 2012) with 5 g L⁻¹ 4-morpholineethanesulfonic acid (MES hydrate SIGMA) added and the pH was adjusted to 6.0 with 5M KOH. Each 160 mL serum bottle received 50 mL of medium and flushed with the same gas mixture as above. The reducing agent solution was prepared in an anaerobic chamber by mixing 2.5g cysteine HCl with 100 mL of anoxic water in a 160 mL serum bottle. The bottle was stoppered in the anaerobic chamber (Chemglass, Inc), crimped outside and flushed for 5 minutes at 5 psig with argon then pressurized to 20psig. The bottle was autoclaved for 20 minutes on a liquid cycle and stored in the dark at room temperature. At least 12 hrs before use, 1YCM and mRCMf were reduced aseptically with 1 and 2 mL per serum bottle, respectively, of reducing solution.

Preparation of Coating Suspension

A mid-log cell suspension (OD₆₀₀~1.3) was transferred to two autoclaved 50 mL Oak Ridge centrifuge tubes sealed with silicone gasket caps in the anaerobic chamber. The cells were centrifuged at 4°C for 15 min at 6000g and then the tubes were moved into the anaerobic chamber. The supernatant was decanted, the pellet rinsed by resuspending in 1YCM, and then centrifuged again at 4°C for 15 min at 6000g. After decanting the supernatant in the anaerobic chamber, the pellet was mixed by vortexing until homogenous. Coating emulsion was prepared by adding cell paste to 1YCM at 30% (v/v) and applied at 6.5 g DCW/m². Cell loadings were estimated using calibration curves that yielded 363mg dry cell weight OTA1 L⁻¹ OD₆₀₀⁻¹ and 1.9E8 CFU OTA1 mL⁻¹ OD₆₀₀⁻¹ (data not shown).

Coating of Paper Substrate

3MM chromatography paper (Whatman) was prepared by cutting into strips (2×10 or 2×5 cm, 335 µm thick) wrapped in aluminum foil, autoclaved for 20 minutes (dry cycle), and stored in the anaerobic chamber. The coating solution was applied by pipette extrusion by evenly distributing 100 µL of coating suspension per 5 cm² of substrate surface area in the anaerobic chamber (5%H₂/balance Ar, 25°C, 48% relative humidity). Only half the substrate was coated so that the coated portion would remain above the liquid level when the tube was horizontal on the shaker at 37°C.

Preparation of Batch Tube Gas Absorber/Reactors

Coated paper substrates were immediately placed into autoclaved Balch tubes (~26mL working volume) and hydrated as the experiment dictated. For suspended cell studies, the same volume of coating solution was added directly to the tube without a paper support and additional medium was added. Tubes were stoppered (Geo-Microbial Technologies, Inc.) in the anaerobic chamber, removed from the chamber, capped and crimped with aluminum seals. The tubes were then flushed with syngas mixture for 2 minutes at 5 psig, then vented to atmospheric pressure. The tubes were then placed horizontally with the paper substrate vertically oriented in a shaker (Infors-HT, Multitron Standard) with a 25mm diameter of rotation at 37°C. A single gas sample was taken from each tube to calculate the consumption rate. Care was taken to ensure data was taken before 2/3 of the CO was consumed (normally

after 3 to 5 hours) as further incubation demonstrated that the reactivity deviated from linearity due to decreased syngas driving force. Production data was obtained by making 30 tubes that at each time point were sampled for gas, then sacrificed to withdraw the liquid sample.

Determination of the cell adsorption capacity and optimal coating concentration of the 3MM cellulose fibers was accomplished by rinsing freshly prepared biocomposites and putting the paper strips upright into horizontal tubes on the shaker at 100 rpm and 37°C. After 3.5h of shaking the tubes placed into the anaerobic chamber and the biocomposites moved to new tubes with fresh medium and the tubes containing the original medium resealed. The two sets of tubes were then capped, crimped, flushed and placed in the shaker for 17hrs before assaying gas consumption.

Gas Analysis

Headspace gas analysis (H₂, O₂, N₂, CO, and CO₂) was performed using an Agilent 7890 gas chromatograph containing a Supelco 60/80 mole sieve 5A column (6' × 1/8" × 2.1mm SS) and a thermal conductivity detector. The GC was fitted with a custom sample tube to allow direct injection of the 1 mL gas sample from the gas tight syringe (SGE Analytical Science) into the 10 µL sample loop. Argon was the carrier gas at 20 mL/min for 1.5 minutes then increased to 32 mL/min and held for 3.4 min. Oven temperature was held at 170°C for 1.5 min then a ramp of 75°C/min to 240°C and held for 3.4 min. Total run time was 5.9 minutes. Injector and detector temperatures were 160°C and 250°C, respectively. Peak areas were scaled relative to the unconsumed nitrogen peak knowing the initial amount of N₂ and converted to moles consumed per hour.

Liquid Analysis for Ethanol and Acetate

Liquid samples (~1 mL) were removed from anaerobic tubes and frozen in microcentrifuge tubes at -20°C. When assayed, the micro-centrifuge tubes were thawed and centrifuged at 13,000 rpm for 5 min and 150 µL of the supernatant was added to 37.5 µL of 2.5% (m/v) m-phosphoric acid in a fresh tube. The samples were then mixed by vortexing and 120 µL was added to 2 mL amber vial with septum tops (Fisherbrand) with target micro-serts (National Scientific). The vials were analyzed with an Agilent 7890A gas chromatograph using a J&W scientific PEG acid modified column 30m × 0.25 mm ID with a 0.5µm film (DB-FFAP 122-3233), a flame ionization detector, and an autoloader. Oven temperature was held at 75°C for 1 min then a ramp of 25°C/min to 200°C and held for 5 min. The inlet and detector temperatures were both 250°C. The hydrogen flow rate was 40 mL/min and air was 400 mL/min. The injection was 0.5µL with at 10:1 split ratio sent to the column with argon as the carrier gas.

Samples designated for enhanced recovery were upended 25 times over the course of 1 minute ensuring the liquid passed over the coated portion of the biocomposite. Before beginning this process to mimic a falling film, the medium was completely clear. After rocking, much of the cells came off into the medium making it very turbid.

Biocomposite Characterization

Images of *C. ljungdahlii* OTA1 biocomposites were taken at the Analytical Instrumentation Facility (AIF) at NC State University using methods described previously (Bernal et al., 2014).

Static film thickness was estimate by a vertically oriented micrometer (Mitutoyo Absolute) with one end of the 3MM chromatography paper sitting in tap water for increased conductivity. An ohmmeter was attached to the plunger of the micrometer and the wetted biocomposite. Thickness measurements were taken when the values changed dramatically on the ohmmeter indicating a complete circuit.

Results and Discussion

SEM images of the microstructure of *C. ljungdahlii* paper coatings are shown in Figure 1. Initial coatings of 11 g m^{-2} nominal surface area showed, as expected, a loss of macroporosity. Decreasing the loading to 4.4 g m^{-2} reduced blocking of the paper pores. Some of the cells are concentrated in low lying areas and some attach to the tops of the cellulose fibers. These images were used to approximate the concentration of the cells that would coat the paper surface. In this initial study on reduced power input, no adhesive latex polymer binders were used to bind the cells to the 3MM paper in contrast to our previous latex-coated paper studies (Bernal et al., 2014; Gosse et al., 2012).

We then investigated the cell adhesion by measuring CO absorbing activity. We compared consumption data on the biocomposite in a fresh tube after rinsing and with the medium left behind. Figure 2 shows that at the higher cell loadings (4.4 and 5.4 g m^{-2}) there was significant reactivity remaining in the liquid phase after removing the biocomposite. At the lowest two loadings, 4.4 and 6.5 g m^{-2} , there is minimal reactivity remaining in the liquid phase after the biocomposite is removed. The loading of 6.5 g m^{-2} was chosen for further experiments.

Previous 1.54 cm^2 *C. ljungdahlii* OTA1 paper biocomposite work hydrated the cells immediately after coating with 10 mL of minimal medium in a vertical Balch tube (Gosse et al., 2012). By placing the tubes horizontally, a larger surface area could be in contact with the gas phase and reducing the medium volume did not affect the CO absorption reactivity until a threshold of $\sim 4 \text{ mL}$ per tube was reached. (Figure 3). This was unchanged for coating surface areas of 5 or 10 cm^2 ; the reproducibility of our data for both configurations was high. Subsequent studies used 4 mL of medium added per tube. This decreased the liquid volume by 60% on a reactor volume basis, and 69% and 85% on a coating surface basis for the 5 cm^2 and 10 cm^2 biocomposite. The medium volume was decreased to only 15% of total reactor volume while the CO consumption per m^2 of paper was intensified by ~ 14 fold over that previously reported by Gosse et al, 2012 (6.6 versus $\sim 90 \text{ mmol CO m}^{-2} \text{ h}^{-1}$).

As expected, a comparison of the liquid volumes (4 , 6 , 8 mL) after the biocomposite had consumed 100% of the CO showed the greatest increase in secreted product concentrations at the lowest liquid volume. After normalizing the data to the amount of gas initially present and subtracting the argon headspace control, 4 and 6 mL liquid volumes had 80% and 26% ,

respectively, higher total product concentration than 8 mL. The final secreted products in the 4 mL tube were 4.3 ± 0.21 g/L with a 0.052 ± 0.0054 ethanol/acetate ratio (triplicate determinations, mol/mol). This is a ~150% yield from the initial amount of CO which is the only carbon source provided in the medium 1YCM. That indicates some unfermented fructose remained surrounding or inside the cells through the rinse step. However, it is the same for all cases investigated. The ethanol/acetate ratio is much lower than reported elsewhere in the literature for research focusing on optimizing ethanol production (Gaddy et al., 2007; Yasin et al., 2015).

A comparison of the reactivity of biocomposites and the same number of cells in suspension in the horizontal tubes showed that the biocomposite outperformed the CO absorption rate of suspended cells at all the shaking speeds <100 rpm (Figure 4). At 0 rpm, the biocomposite outperforms the cells in suspension by >5 fold. Using equations 3-4, the power requirements of the different shaking conditions (except 0 rpm) can be compared. At 25, 50, 75, and 100 rpm the power requirements are 0.21, 1.2, 3.6, 7.7 W m⁻³, respectively. Comparing the biocomposite at 100 vs 25 rpm, a reduction of the power requirement by 97% reduced the consumption rate by only 36%.

It can be seen that both the biocomposite and the cells in suspension have reactivity that are dependent upon the power input (shaking speed). The reason is obvious for the planktonic cells. However, the biocomposite's reactivity is dependent upon shaking speed likely because of the increased motion of the medium into and out of the paper pores providing the cells with more nutrients and removing secreted products from the cells' microenvironment. Figure 4 shows that the cells in suspension are >50% more dependent on the power input (steeper fit) while having a lower reactivity. It is interesting to note that the least squares fit predicts a crossover at ~250 rpm where suspended cells will outperform a biocomposite. The energy requirement at that level is 89 W m⁻³, >11 fold higher than at 100 rpm, and >420 fold higher than the power input at 25 rpm.

A time course experiment was performed to compare gas consumption along with secreted product production at 50rpm. The gas consumption results in Figure 5a show the biocomposite outperforming the same number of suspended cells by more than 2 fold. The liquid product production results are not as dramatic (Figure 5b). Only acetate production is shown due to a negligible amount of ethanol produced (<0.1 g L⁻¹). Before ~2.5 hours, more acetate is found in the liquid phase of the suspended cells. After that point, there is more acetate in the liquid phase of the biocomposite tubes, but much less than what would be expected. The liquid products may be concentrated in the pores of the paper substrate. In a flowing thin film system this diffusion resistance of products out of the biocomposite pores can be minimized. The simple shaking tube system demonstrates a biocomposite final acetate titer of 1.8 g L⁻¹ and an acetate production rate of ~0.5 g L⁻¹ h⁻¹.

A separate experiment compared the specific consumption and production rates for suspension and different nominal surface areas of biocomposite papers. Table I shows that the consumption rate per area and per g_{cells} almost constant across the 3 biocomposite conditions which demonstrates the simplicity of scaling. Additionally, the 5 cm² biocomposite has almost identical performance to the suspended cells even though that

biocomposite has half the number of cells. The reactivity per volume of liquid (what you have to process) or reactor volume (what you have to pay for) increases linearly with coated surface area. Finally, because adding more biocomposite surface area increases interfacial area, a , the apparent mass transfer coefficient, $k_L a_{app}$, increases linearly as well without the input of any more power than required for composite material fabrication.

The liquid product production results from the experiment in Table I are shown in Table II along with data from the literature for comparison. As before, though the biocomposite absorbed ~2 times more CO, it produces the same amount of secreted products. Even with enhanced recovery (mixing) to mimic a falling film (flow of secreted products out of the paper pores), the biocomposite outperforms the same number of suspended cells by 22%. More work is needed to optimize the cellulose paper structure so the biocomposite product is not trapped in fluid in the paper pores. It can be seen that the secreted product per volume productivity scales approximately linearly with coating area. The productivity per area values are approximately the same across all the enhanced recovery samples. The highest nominal surface area biocomposite's specific volumetric production rate exceeds almost all the examples but for a high pressure, optimized media, cell recycle CSTR (Gaddy et al., 2007). The specific production rate per unit area also outperforms the limited examples where the calculation can be made but from Coskata, Inc. (Tsai et al., 2012). Interestingly, the two rates are similar. The Coskata method involves a membrane with imbedded cells; not unlike a biocomposite. Critical for success of a biocomposite system at scale will be the achieving similar long term biocatalyst stability results in a continuous system as was previously shown in batch (Gosse et al., 2010; Gosse et al., 2012)

The data from Figures 3, 4, and Table I were manipulated using equation 2 in order to obtain a minimal estimate of the mass transfer coefficient, $k_L a_{app}$. The results were plotted in relation to their calculated power requirements and compared with other reactor types in Figure 6. (Arjunwadkar et al., 1998; Boodhoo et al., 2008; Bredwell et al., 1999; Garcia-Ochoa and Gomez, 2009; Gourich et al., 2006; Kraakman et al., 2011; Kreutzer et al., 2005; Linek et al., 2005; Markopoulos et al., 2007; Pirouzi et al., 2014) All results for $k_L a_{app}$ generated experimentally are for CO. They were changed into mass transfer coefficients for O₂ for proper comparison using a ratio of the square root of the diffusivities according to penetration theory (Danckwerts, 1951).

In order to confirm that the results are reasonable, it was determined that 264 rpm shaking speed would be ~100 W m⁻³ and the $k_L a_{app}$ was calculated for cells in suspension and plotted (see inverted triangle, Figure 6). This gives confidence that our modifications to the power calculation method and $k_L a_{app}$ are reasonable as it falls just above the trickle bed regime which is expected because of the small liquid phase volume being spread into a thin film on the tube's inner surface.

The biocomposites' $k_L a_{app}$ in this simple system is lower than some of the $k_L a$ values reported for other bioreactors. However, $k_L a_{app}$ calculated here is a minimal estimate; the actual value will be higher. Our data indicates a $k_L a_{app}$ for CO of ~100 h⁻¹ with a power requirement that is 1 to 4 orders of magnitude lower than reported for other bioreactor types and matches or exceeds mass transfer rates reported for syngas fermentations (Munasinghe

and Khanal, 2010; Yasin et al., 2015). Having solute consuming microorganisms will increase the observed mass transfer coefficient if mass transfer is limiting (Garcia-Ochoa and Gomez, 2009). By using the nominal reactive paper surface area and measuring the film thickness, k_L can be estimated. The static liquid film thickness on top of the paper was measured to be 130 μm . Assuming, as a first approximation, a flat film over the cells and no bubbles, the interfacial area is $\sim 8000 \text{ m}^2$ and k_L is therefore $\sim 2\text{E-}6 \text{ m s}^{-1}$. A first approximation for a static film is the membrane equation, $k_L l/D=1$ (Cussler, 2009) where l is the membrane thickness or in this case, the film thickness. Using the diffusivity of CO in water, k_L is $\sim 2\text{E-}5 \text{ m s}^{-1}$ or a full one order of magnitude higher than what we observe experimentally. So gas-to-liquid mass transfer is not limiting in this configuration. The system is limited by the cells' metabolism (see also the specific uptake rates in Table I). The biocomposite rates are below the maximum reported in literature for dilute suspended cells, 14 vs 35 $\text{mmol g}_{\text{DCW}}^{-1} \text{ h}^{-1}$. The suspended cell maximum uptake rate is likely not reached because some cells are entrapped in the interior of the paper and all of the substrate is consumed by the cells on the surface. The limitation is mass transfer within the structure of the biocomposite. Thinner paper surface coatings or thinner papers with uniformly coated cellulose fibers should diminish this effect.

A continuous system could be constructed of multiple parallel biocomposite tubes mounted inside larger tubes to intensify volumetric reactivity ($\text{mmol L}_{\text{Reactor}}^{-1} \text{ h}^{-1}$). This design may initially appear similar but is conceptually different from hollow fiber bioreactors. In a biocomposite reactor, the cells would be immobilized in nonwoven tubes prior to reactor module construction and there is no bulk liquid phase either inside or outside the lumen. The minimal liquid volume moves within the paper pores (within the nonwoven tube walls) while the gas in contact with a thin liquid film will move on both sides of the biocomposite (Figure 7).

Conclusions

Carbon monoxide consumption as well as acetate production was investigated in batch system in suspension and using a paper-based biocomposite. The biocomposite outperformed the same number of cells in suspension in terms of specific rates of gas consumption, specific acetate production rate, and acetate titer. Methods were presented for estimating the mass transfer coefficient and power required and the biocomposite data compared favorably with stirred tank bioreactors at 1 to 4 orders of magnitude less power. Stabilization of *C. ljungdahlii* OTA1 paper biocomposites by engineered adhesion, optimizing paper, coating microstructure, cellulose fiber coverage and dry stabilization are currently being investigated by our group. The critical design parameters and intensification shown here as a proof of concept in combination with previous work on biocatalyst longevity show the potential of thin film biocomposite reactor designs for GTL processes utilizing microbes.

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Figure 1.

SEM images of 3MM paper substrates and biocomposite coatings showing concentrated cell patches and cells adsorbed on top of cellulose fibers. (a) Bare Whatman 3MM chromatography paper (b) Coating of $\sim 11 \text{ g m}^{-2}$ (c) High magnification image of $\sim 11 \text{ g m}^{-2}$ multilayered cell coating (d) Cell loading of $\sim 4.4 \text{ g/m}^2$ (e, f) High magnification images of $\sim 4.4 \text{ g m}^{-2}$ cell coating.

Figure 2.

Comparison of reactivity of *C. ljungdahliae* OTA1 rinsed off of a biocomposite as a function of different cell loadings by adsorption. Cells removed from biocomposite by shaking at 100 rpm with transfer to a new tube with gas flushing. Both duplicates shown.

Figure 3.

Comparison of CO uptake reactivity in horizontal Balch tubes with different liquid medium volume. Each data point per gram dry cell weight or per m² of coating area. Reactivity stays elevated until liquid medium volume is ~<4mL or ~15% of reactor volume. Triplicate determinations, n=3. 20 cm² 3MM paper substrate with a 10 cm² coated area shown. Liquid phase is depicted on the bottom of the horizontal tubes. Shaking agitation depicted by arrow.

Figure 4. Comparison of CO reactivity versus shaking speed for a biocomposite and the same number of cells in suspension in 4 mL of medium in horizontal tubes. Triplicate determinations.

Figure 5.

A time course comparing both gas consumption (a) and liquid product production (b) for biocomposites (circles) and suspended cells (diamonds) at 50rpm. Triplicate determinations. The dotted line along the top of (a) show the total initial CO present. Both replicates of the initial coating solution are shown as crosses.

Figure 6.

Comparison of mass transfer coefficient with power input. Symbols are: open triangle-stirred tank (Arjunwadkar et al., 1998; Boodhoo et al., 2008; Bredwell et al., 1999; Garcia-Ochoa and Gomez, 2009), open circle-bubble column (Linek et al., 2005), closed diamond-trickle bed reactor (Bredwell et al., 1999; Kraakman et al., 2011), closed circle-airlift reactor (Bredwell et al., 1999; Gourich et al., 2006; Loubiere et al., 2009; Pirouzi et al., 2014), cross hatch-monolith or microchannel (Kraakman et al., 2011; Kreutzer et al., 2005), black squares-3MM paper biocomposites. Inverted filled triangle is test case to confirm power calculations. All data is presented in terms of a mass transfer coefficient for O₂.

Figure 7.

Comparison between a hollow fiber and biocomposite reactor. In hollow fiber reactors the membrane separates the gas and liquid phase with the biofilm forming in the shell or bulk liquid phase. The biocomposite has concentrated cells embedded within the tube walls with gas on both sides with the liquid moving through the paper pores and over the surface of the biocomposite in a thin liquid film that minimizes mass transfer resistance.

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Table 1
CO consumption and mass transfer parameters (26 mL reactor volume, 4 mL medium)

Condition	Cell mass (mg)	Nominal coating surface area (cm ²)	CO Consumption Rate ± SE (n=3)				$k_L a_{\text{apparent}}$ (h ⁻¹)
			mmol g _{DCW} ⁻¹ h ⁻¹	mmol L _{Liquid} ⁻¹ h ⁻¹	mmol L _{Reactor} ⁻¹ h ⁻¹		
<i>Suspended</i> <i>Biocomposite</i>		mmol m ⁻² h ⁻¹					
	6.5	N/A	N/A	4.6 ± 0.085	10 ± 0.2	1.5 ± 0.03	26
	3.3	5	90 ± 13	14 ± 2.0	11 ± 1.6	1.7 ± 0.24	30
	6.5	10	87 ± 2.0	13 ± 0.31	22 ± 0.5	3.3 ± 0.08	56
	13	20	94 ± 2.4	14 ± 0.37	47 ± 1.2	7.2 ± 0.18	116

Table II

Production characteristics and reactor comparison

Reactor Type	Notes	Ethanol: Acetate (mol/mol)	C ₂ production rate $\pm$ SE (n=3)		Ref
			mmol L _{Liquid} ⁻¹ h ⁻¹	mmol m ⁻² h ⁻¹	
<i>Suspended cells</i>	Batch	0.001	8.2 $\pm$ 0.04	N/A	This work
<i>Biocomposite</i>	10 cm ² batch	0.001	7.8 $\pm$ 0.4	31 $\pm$ 1.6	This work
	10 cm ² batch Enhanced recovery	0.001	10 $\pm$ 0.5	40 $\pm$ 2.1	This work
	5 cm ² batch Enhanced recovery	0.003	5.4 $\pm$ 1.0	43 $\pm$ 7.8	This work
	20 cm ² batch Enhanced recovery	0.002	23 $\pm$ 0.6	46 $\pm$ 1.3	This work
<i>STR</i>	Continuous Cell recycle	21	1.95	N/A	(Phillips et al., 1993)
<i>STR</i>	Continuous Cell recycle	2.2	2.22	N/A	(Liu et al., 2014)
<i>STR</i>	Continuous gas Batch liquid 100 L pilot	5.2	0.79	N/A	(Kundiyana et al., 2010)
<i>STR</i>	High Pressure Optimized media Cell recycle Continuous	12	240	N/A	(Gaddy et al., 2007)
<i>Bubble</i>	Cell recycle Continuous	3.0	9.7	N/A	(Richter et al., 2013)
<i>Monolith</i>	Cell recycle Continuous gas Batch liquid	2.1	3.2	6.6	(Shen et al., 2014b)
<i>HFMR</i>	Cell recycle Continuous gas Batch liquid	1.5	5.1	29	(Shen et al., 2014a)
<i>HFMR</i>	Continuous Cells embedded in membrane	3.3	5.4	51	(Tsai et al., 2012)