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BIOCATALYSIS

A versatile method for preparation of hydrated microbial–latex biocatalytic coatings for gas absorption and gas evolution

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Abstract We describe a latex wet coalescence method for gas-phase immobilization of microorganisms on paper which does not require drying for adhesion. This method reduces drying stresses to the microbes. It is applicable for microorganisms that do not tolerate desiccation stress during latex drying even in the presence of carbohydrates. Small surface area, 10–65 μm thick coatings were generated on chromatography paper strips and placed in the head-space of vertical sealed tubes containing liquid to hydrate the paper. These gas-phase microbial coatings hydrated by liquid in the paper pore space demonstrated absorption or evolution of H_2, CO, CO_2 or O_2. The microbial products produced, ethanol and acetate, diffuse into the hydrated paper pores and accumulate in the liquid at the bottom of the tube. The paper provides hydration to the back side of the coating and also separates the biocatalyst from the products. Coating reactivity was demonstrated for *Chlamydomonas reinhardtii* CC124, which consumed CO_2 and produced 10.2 ± 0.2 mmol O_2 m^{-2} h^{-1}, *Rhodopseudomonas palustris* CGA009, which consumed acetate and produced 0.47 ± 0.04 mmol H_2 m^{-2} h^{-1}, *Clostridium ljungdahlii* OTA1, which consumed 6 mmol CO m^{-2} h^{-1}, and *Synechococcus* sp. PCC7002, which consumed CO_2 and produced 5.00 ± 0.25 mmol O_2 m^{-2} h^{-1}. Coating thickness and microstructure were related to microbe size as determined by digital micrometry, profilometry, and confocal microscopy. The immobilization of different microorganisms in thin adhesive films in the gas phase demonstrates the utility of this method for evaluating genetically optimized microorganisms for gas absorption and gas evolution.

Keywords Latex coating immobilization on chromatography paper · *Chlamydomonas* · *Rhodopseudomonas* · *Clostridium* · *Synechococcus*

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Introduction

Whole-cell biocatalysts immobilized onto insoluble supports are used for biosensors, environmental remediation, biofuel production, and biosynthesis of chiral chemicals

[2, 4, 8, 29]. Cell immobilization on membranes or porous supports can concentrate (intensify) biological reactivity and stabilize microbes [10, 13, 14, 17, 33]. Trickle bed and biocatalytic membrane reactors (MBRs) are used in many industries [16]. They are used for generation of H_2, in recycling of gas-phase carbon emissions (CO_x) to fuels, for sulfur (SO_x) and nitrogen (NO_x) removal from gases, and in biodesulfurization of petroleum. Interest in microbial biocatalysts as reactive biofilters that absorb gases (CO, CO_2, CH_4, H_2, NO_x, SO_x) or produce useful gases (O_2, H_2, CH_4) will undoubtedly increase with emphasis on low-power-input large-scale processes such as conversion of CO-rich synthesis gas (syngas) and capture of waste greenhouse gases to recycle into biofuels [1, 3, 7, 20, 24, 34]. For this reason, there is much interest in the efficiency of gas–liquid–microbe mass transfer in multiphase bioreactors, energy-efficient methods to generate microbubbles, thin-film bioreactors, and optimization of the microstructure of multilayered structured biocoatings of very high density, reactive, but *nongrowing* microorganisms for large-scale biocatalysis with low power input for mass transfer [12, 17]. All of the above reactors seek to minimize power input for gas–liquid–microbe mass transfer [1, 3, 24, 34]. Entrapping immobilized microorganisms in thin coatings on inexpensive flexible substrates may also be useful as "biomimetic leaves" for uniform illumination of multiple layers of photosynthetic microbes to absorb or produce gases while optimizing light trapping [17].

Microbial latex coatings differ significantly from natural biofilms or cells immobilized in hydrogels. There are fundamental limitations with cell immobilization by growing biofilms to colonize support media in trickle beds, packed beds, biofilters, and membrane bioreactors. Natural biofilms have limited control of cell concentration (intensification) and poor control of film thickness (to reduce the diffusion path), and only support microbes that will adhere to, colonize, and form an extracellular matrix. In addition, natural biofilms release cells, leading to reactor plugging. Techniques which rely on natural or synthetic hydrogel polymers to entrap or immobilize microorganisms suffer from similar problems, and their structure may not remain stable, resulting in release or outgrowth of cells. Hydrogel pores are not stable when dried and therefore cannot preserve the reactivity of the entrapped microbes in dry form.

For syngas fermentation, due to the low solubility of H_2 and CO at low pressures, gas–liquid mass transfer rates are very low, even for well-mixed stirred tank bioreactors (STRs) even with microbubble aeration [1, 3, 31]. This process would greatly benefit from a low energy input, high gas volume to liquid volume ratio (>1.0) design to significantly increase gas mass transfer to the microbes; however, evaluation of differing reactor designs on laboratory scale is difficult. For example, the lack of a standardized method to screen microbes for conversion of syngas (a mixture of CO, CO_2, N_2, and H_2) to ethanol, acetate, and other fuels often frustrates direct comparison of the biocatalyst reactivity reported by various investigators per unit of gas surface area in contact with the microorganisms [1, 3, 7, 18, 20, 24, 25, 28, 34]. Increasing the agitation power input in STRs to increase syngas bubble surface area using high-shear microbubble generators or methods to increase bubble residence time and thus the mass transfer rate may not be economically feasible for very large-scale gas absorbers. As a result, a variety of membrane bioreactors (MBR) with reduced power input for efficient mass transfer have been investigated [1, 7, 20].

In the case of photosynthetic biohydrogen gas production, not only are many candidate microbial systems being investigated (algae, purple nonsulfur bacteria, cyanobacteria), but also a wide variety of bioreactors have been proposed. The most important features are effective contact of the gas phase with the photoreactive microbes and providing sufficient, uniform illumination at high cell density. Examples are tubular or sheet photobioreactors, bubble columns, biofilm coated-membrane bioreactors (sheet or hollow fiber), supported biofilms (rotating biological contactors, stacked array bioreactors, horizontal array bioreactors), trickling packed beds (utilizing a variety of packing materials), and agitated anaerobic bioreactors. These systems all require determination of the effect of microbe concentration, light scattering, and illumination uniformity on productivity. In the case of cell immobilization, key elements such as the pore structure of the immobilization matrix, the thickness of the microbial layer, and the extent to which the cells are retained on the support in the bioreactor are often not accurately measured or reported. Combined, these differences result in difficulty in directly comparing the specific reactivity of many of these experimental systems.

For conversion of sparingly soluble gases, such as H_2 and CO (whose solubility in water is 60 and 4 % that of oxygen, respectively, on a mass basis), the reactivity and product yield of these systems cannot be optimized by cellular engineering alone. Screening for the rate of gas conversion or production and conversion yield of genetically altered microorganisms must also include a small-scale bioreactor configuration to efficiently contact a high concentration of microbes with gaseous substrates. This is because, at low cell concentrations, the overall reaction rate of these processes is kinetically limited, whereas at high cell concentrations, the kinetics become mass transfer limited. Screening organisms in the laboratory under conditions that have little similarity to an industrial reactor or absorber may not select for optimal biocatalyst traits. It is, however, challenging to scale down reactors and immobilization methods. For this reason, there is much interest in a

simple laboratory method for increasing the efficiency of gas–liquid–microbe mass transfer for evaluation of microbes suitable for thin-film bioreactors. This includes optimization of the microstructure of structured (multilayered) coatings or ordered arrays with a very high density of reactive but nongrowing microorganisms for engineering large-scale bioabsorbers or biophotoabsorbers with low power input for mass transfer and uniform cell illumination [12, 17].

A simple, high cell density, inexpensive, nontoxic, adhesive, nanoporous immobilization method which can be used to evaluate thin films of many different microorganisms in a simple miniature gas-contacting reactor would greatly improve screening of engineered microbes for biofilter, photobioreactor, or energy applications. The method must have controlled microbial deposition and adhesion, and easily measured surface area, and allow accurate determination of the number of microorganisms per unit surface area. Such a laboratory method could be used to more accurately determine and predict the best combination of microbe and large-scale bioreactor configuration for optimal gas–liquid–microbe contact, illumination, and conversion efficiency. To be most efficient, a microbial gas-phase biocatalyst should be a thin nanoporous insoluble coating that is adhesive to the (porous or nonporous) substrate when hydrated, with nutrient limitation or genetic alteration of the immobilized microorganism to prevent outgrowth (reactor plugging). This coating approach allows uniform distribution of a very high density of microbes in a thin coating that is either in a water-saturated gas phase, is self-hydrating from within, or is hydrated by a porous network behind the coating in order to stabilize microbial reactivity without desiccation. For rapid screening of a variety of microbial systems in the gas phase (for example, the head-space in a tube above the liquid phase), the surface area of the coating should be as large as possible and the gas-phase volume should be small (<25 ml). Similarly, any thin coating immobilization method useful in the gas phase must also be stable when submerged to directly compare conversion of substrates dissolved in the bulk liquid phase to the reactivity of hydrated gas-phase coatings.

Herein we describe a paper coating method using a nontoxic, inexpensive, commercially available latex binder used for paper coating and paints. This emulsion contains latex binder mixed with microorganisms and osmoprotectant carbohydrates. The emulsion is coated onto dry chromatography paper, resulting in adhesion between the cells and the cellulose fibers and thus microbe entrapment in a nanoporous layer by polymer particle wet coalescence without drying. The utility of this approach is demonstrated for immobilization of four different microbial systems (eukaryotes and prokaryotes) of different size, which either consume or generate gases. This method may be broadly applicable to many different microorganisms to fabricate inexpensive small-scale supported membrane tube bioreactors (SMBs) for screening.

Reactivity data for coatings of *Chlamydomonas reinhardtii* CC124 (eukaryotic microalgal aerobic phototroph), *Rhodopseudomonas palustris* CGA009 (anaerobic bacterial phototroph), *Clostridium ljungdahlii* OTA1 (anaerobic autotrophic acetogenic ethanologen), and *Synechococcus* sp. PCC7002 (cyanobacterial aerobic phototroph) incubated in sealed tubes are reported. The phototrophic microorganisms chosen are representative of the types of organisms being considered for various renewable energy applications, especially green microalgae. *C. ljungdahlii* has received considerable attention as a biocatalyst for conversion of syngas to ethanol. A number of companies are actively working to commercialize syngas fermentation. All of the organisms in this study depend on gas–liquid phase interactions. *R. palustris* requires a soluble organic electron donor, such as acetate, for H_2 production; *Synechococcus* requires only micronutrients, CO_2, light, and water for O_2 evolution, while *C. reinhardtii* requires micronutrients, CO_2, light, and water for O_2 evolution in addition to possibly requiring acetate. *C. ljungdahlii* uses CO and H_2 as substrates for ethanol and acetate production with net CO_2 production. Of the model microbial systems evaluated here, the purple nonsulfur bacterium (PNSB) *R. palustris* is the only one that does not consume a gas. Under a N_2-free, anaerobic atmosphere such as argon, *R. palustris* produces hydrogen gas. PNSB are being intensively studied for photoassisted production of biohydrogen from waste organics [17, 30].

Materials and methods

Bacterial strains, media, and growth conditions

Wild-type *Rhodopseudomonas palustris* CGA009 was grown in 160-ml glass serum bottles (Wheaton, Millville, NJ) containing 100 ml anaerobic nitrogen-fixing photosynthetic medium with 20 mM sodium acetate [PM(NF) 20ace] and an initial head-space pressure of 1 atm N_2 [17, 18]. Wild-type *Chlamydomonas reinhardtii* CC124 was grown aerobically in a 1-l baffled Erlenmeyer flask containing 250 ml TAP medium with stirring under cool white fluorescent light at 28 °C [19]. Wild-type *Synechococcus* PCC7002 was grown aerobically in 250-ml baffled Erlenmeyer flasks containing 50 ml BG-11 medium at 26 °C with agitation (100 rpm) [32]. *Clostridium ljungdahlii* OTA1 was grown with shaking at 100 rpm in 160-ml glass serum bottles containing 50 ml anaerobic 1YCMf medium and initial head-space pressure of 1 atm artificial

syngas (10 % H_2, 20 % CO_2, 20 % CO, 50 % N_2) at 37 °C [35].

PM(NF)20ace, TAP, and BG-11 were prepared as previously described [17–19, 31, 32]. 1YCM contains (in 910 ml) 1 g yeast extract, 50 ml PETC salts (per l, 20 g NH_4Cl, 2 g KCl, 4 g $MgSO_4{\cdot}H_2O$, 16 g NaCl, 0.4 g $CaCl_2{\cdot}H_2O$, 2 g KH_2PO_4), 10 ml PETC modified trace elements (per l, 2 g nitrilotriacetic acid, 1.3 g $MnCl_2{\cdot}4H_2O$, 0.4 g $FeSO_4{\cdot}7H_2O$, 0.2 g $CoCl_2{\cdot}6H_2O$, 0.2 g $ZnSO_4{\cdot}7H_2O$, 0.02 g $CuCl_2{\cdot}2H_2O$, 0.02 g $NiCl_2{\cdot}6H_2O$, 0.02 g $Na_2MoO_4{\cdot}2H_2O$, 0.02 g Na_2SeO_3, 0.025 g $Na_2WO_4{\cdot}2H_2O$, pH adjusted to 6.0 with 5 M KOH), 10 ml Wolfe's vitamin solution (per l, 0.002 g biotin, 0.002 g folic acid, 0.01 g pyridoxine, 0.005 g thiamine·HCl, 0.005 g riboflavin, 0.005 g nicotinic acid, 0.005 g pantothenic acid, 0.005 g vitamin B_{12}, 0.005 g *p*-aminobenzoic acid, 0.005 g lipoic acid), and 0.2 ml resazurin (5 mg/ml), and 5 g of fructose when making 1YCMf. After mixing, the pH was adjusted to 6.8 with 5 M KOH. Serum bottles (160 ml) containing 45.5 ml 1YCM medium were sealed and flushed with syngas (20 % CO, 20 % CO_2, 10 % H_2, 50 % N_2), then autoclaved. After the bottles had cooled to room temperature, the medium was reduced with 1 ml each of 2.5 % (w/v) cysteine-HCl and 2.5 % (w/v) sodium sulfide by anaerobic aseptic addition. Cultures were started with a 5 % inoculum of actively growing *C. ljungdahlii* OTA1 cells [35].

Latex immobilization

For all cell types, the latex–cell formulations were prepared from wet cell pellets. *R. palustris* CGA009 wet cell pellets were prepared by centrifuging 400 ml culture at 4,424 × *g* and 4 °C for 15 min, suspending the cells in 40 ml PM(NF) medium without acetate and transferring to sterile 50-ml conical tubes, centrifuging at 5,000 × *g* at 4 °C for 15 min, and then pouring off the supernatant. *C. reinhardtii* CC124 wet cell pellets were prepared by centrifuging 45 ml culture for 3 min at 3,000 × *g* and 4 °C, suspending the cells in 40 ml TAP medium and transferring to sterile 50-ml conical tubes, centrifuging at 3,000 × *g* at 4 °C for 3 min, and then pouring off the supernatant. In some cases, TAP medium (500 µl) was added to the pellet in order to sufficiently suspend the pellet for transfer by pipette when making the formulation. *C. ljungdahlii* OTA1 wet cell pellets were prepared under anaerobic conditions by centrifuging 200 ml culture at 6,000 × *g* and 4 °C for 15 min, suspending the cells in 40 ml 1YCM medium and transferring to sterile 50-ml conical tubes, centrifuging at 5,000 × *g* at 4 °C for 15 min, and then pouring off the supernatant. *Synechococcus* CC124 wet cell pellets were prepared by centrifuging 45 ml culture for 3 min at 3,000 × *g* and 4 °C, suspending the cells in 40 ml BG11 medium and transferring to sterile 50-ml conical tubes, centrifuging at 3,000 × *g* at 4 °C for 3 min, and then pouring off the supernatant.

The wet cell pellets were mixed by vortexing (15 s) until homogeneous slurries of cells were obtained. From the suspensions for each cell type, 200 µl was transferred to an Eppendorf tube containing 200 µl SF012 latex (Rhoplex™ SF-012, an organic solvent-free acrylate copolymer latex paint binder, 43.5 % solids, maximum viscosity 300 cP, minimum film formation temperature 0 °C, pH 7–8, prepared without biocides; Rohm and Haas Co., Philadelphia, PA), and the slurry was mixed by vortexing until homogeneous (15 s). Coatings were subsequently prepared by transferring 50 µl of the cell/latex slurry evenly over a scribed 14-mm circle centered on one end of a dry, sterile, folded 3MM chromatography paper template (2 cm × 14 cm) (Fig. 1). Before drying, freshly prepared coatings were wetted with the appropriate growth medium by placing the paper strips into vertical Balch tubes containing 10 ml medium and allowing capillary forces to wick the medium from the bottom of the tube to the top of the paper strip.

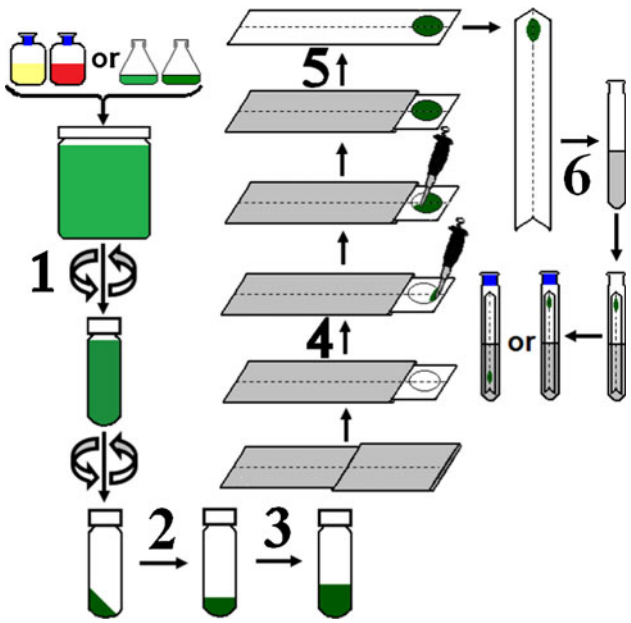
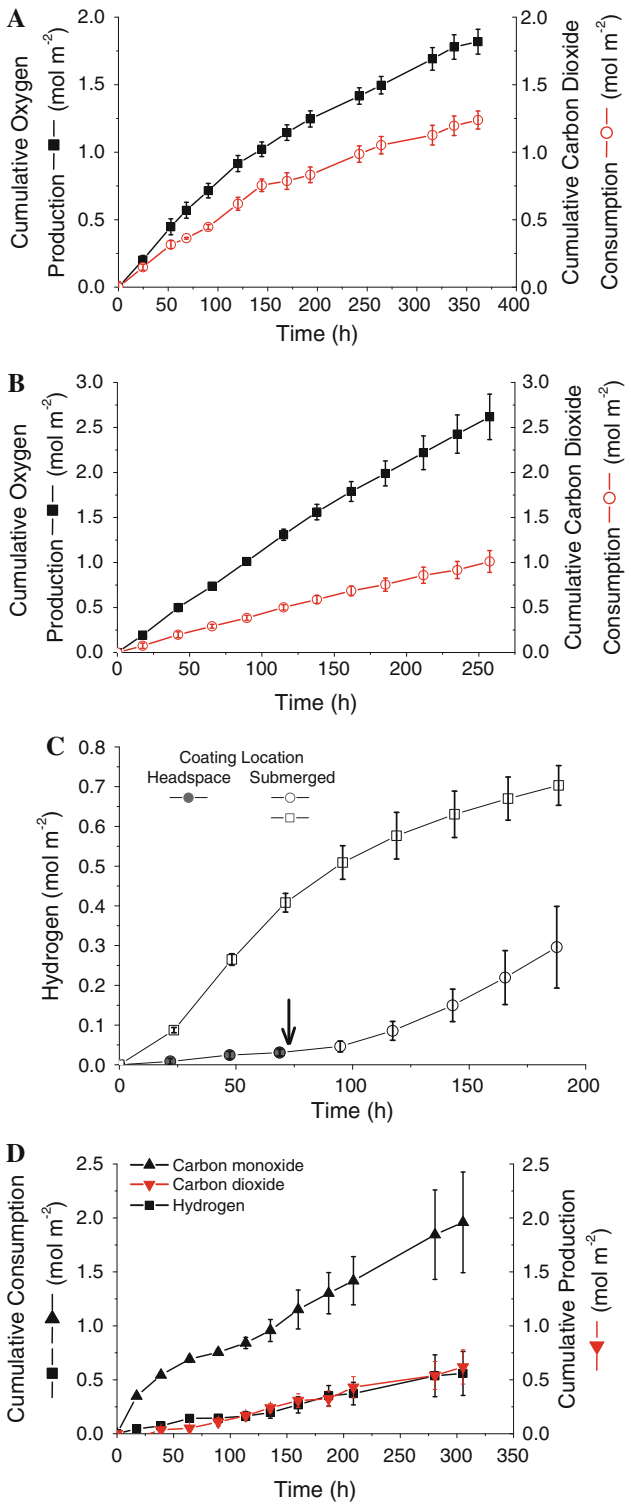


Fig. 1 Latex immobilization method. All coating steps are carried out according to the requirements of the individual organism being immobilized (for example, in an anaerobic chamber for *C. ljungdahlii* coatings). *1* Cells grown and harvested by centrifugation. *2* Cells suspended by vortexing. *3* Latex mixed with cell suspension until homogeneous. *4* Cell formulation applied to demarcated surface of exposed sterile 3MM chromatography paper substrate using a pipette (*gray* area shows 3MM filter paper strip wrapped in two-part foil sleeve for sterility). *5* Cell-coated paper strip folded and removed from sterile foil cover. *6* Cell-coated paper strip inserted into a Balch tube by either submerging the coating in 10 ml medium (with coating pointed down) or leaving the coating in the head-space (coating at the top)



◀ **Fig. 2** Cumulative oxygen or hydrogen evolution and carbon monoxide or carbon dioxide utilization by hydrated paper-immobilized organisms in sealed tubes. Oxygen evolution (*black squares*) and carbon dioxide consumption (*red circles*) by coatings of **a** *Synechococcus* sp. PCC7002 and **b** *C. reinhardtii* CC124. **c** Hydrogen production by *R. palustris* CGA009 coatings either submerged in medium (*open symbols*), or wetted but above the medium (*closed circles*). The arrow indicates when the coating was submerged in medium. **d** Carbon dioxide evolution (*inverted red triangles*), carbon monoxide consumption (*black triangles*), and hydrogen consumption (*black squares*) by *C. ljungdahlii* OTA1 coatings. All values represent means from triplicate experiments ±1 standard deviation of $n = 3$ (color figure online)

Imaging and microstructure analysis

Freshly prepared strips were frozen at −80 °C. The thickness of the chromatography paper, both dry and hydrated, was determined from triplicate frozen coatings using a digital micrometer with rounded tip (model ID-C112GEB; Mitutoyo USA Corporation). Coated paper surface topography and coating thickness were also measured by attaching the 3MM chromatography paper to clean glass slides to prevent the surface from curling under the stylus force (3 mg force) and measuring using a surface profilometer (Dektak D150, Veeco, Plainview, NJ) equipped with a 12.5 μm radius and Dektak v9 software.

Hydrated 3MM chromatography paper microstructure was determined from deconvoluted *z*-plane images obtained by confocal laser scanning microscopy (CLSM) using an Olympus BX-61 optical microscope (Olympus America, Center Valley, PA) equipped with transmitted-fluorescence modes and a 515-nm argon-ion laser (CVI Melles Griot, Albuquerque, New Mexico, USA). A stack of 50 CLSM images, 4 μm apart in the *z* direction perpendicular to the paper plane, were taken to a total depth of ~200 μm into the paper. ImageJ software (National Institutes of Health, Bethesda, Maryland) was used to reconstruct the topographic profile of the samples. The Tikhonov–Miller algorithm was used for image deconvolution, noise reduction, and improving image quality.

Head-space gas absorption or evolution

The coated chromatography paper was immediately placed into vertical Balch tubes containing 10 ml of the appropriate medium without waiting for the latex coating to dry; the tubes were sealed and flushed for 5 min to achieve the required head-space composition (Table 1). The paper strip could be placed into the tube either with the coating at the top (above the liquid phase) or with the coating at the bottom, submerged below the liquid surface, depending on the microbial system being evaluated. When the coating was at the top of the tube, the uncoated end of the paper strip was submerged in the 10 ml liquid phase and the entire chromatography paper was completely hydrated in <5 min by wicking. The coatings remained adhesive and stable during paper hydration. The latex coatings were not observed to penetrate through the 3MM chromatography

Table 1 Model microorganisms used in this study to generate the biocatalytic coatings and the specific reactor conditions for each

Organism	Initial reactor atmosphere	Gas substrates	Liquid substrates	Gas products
Chlamydomonas reinhardtii CC124	20 % CO_2, 80 % N_2	CO_2	Acetate	O_2
Rhodopseudomonas palustris CGA009	100 % Ar	N/A	Acetate	H_2, CO_2
Clostridium ljungdahlii OTA1	10 % H_2, 20 % CO 20 % CO_2, 50 % N_2	CO, H_2	N/A	CO_2
Synechococcus sp. PCC7002	20 % CO_2, 80 % N_2	CO_2	N/A	O_2

N/A not applicable

paper substrate during paper hydration. All tubes were vented using a water trap back to 1 atm. The *R. palustris* coatings were evaluated in paper strip configurations (above liquid phase, completely submerged). In order to duplicate the method as previously reported [18], *R. palustris* coatings were dried at 30 °C and 60 % relative humidity for 1 h prior to placement into Balch tubes. *Synechococcus* and *Chlamydomonas* coatings were incubated under fluorescent light with 68 μmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) illumination without shaking at 28 °C, and *R. palustris* was incubated under incandescent light with 24 μmol photons m^{-2} s^{-1} PAR without shaking at 30 °C. *C. ljungdahlii* was incubated in the dark at 37 °C without shaking.

Head-space analysis

A Hewlett Packard 7890A gas chromatograph containing a Supelco 6′ × 1/8″ ID 60/80 mol sieve 5A porous mesh polymer-packed stainless steel column and a thermal conductivity detector was used for head-space analysis of CO, CO_2, O_2, N_2, and H_2. Argon was used as the carrier gas at flow rate of 36 cc/min with injector, oven, and detector temperature settings of 160, 160, and 250 °C, respectively.

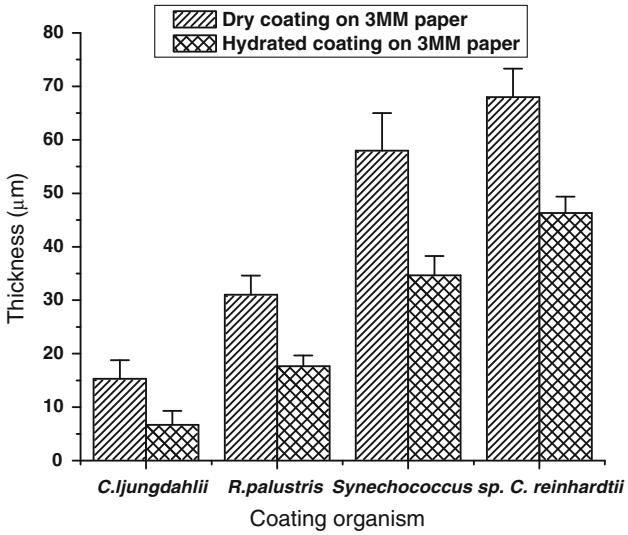


Fig. 3 Latex coating thickness determined on frozen coatings for four different microorganisms on 3MM chromatography paper. Dry coatings were not hydrated prior to freezing at −80 °C. Coatings were immediately hydrated after latex deposition (without air drying) by the appropriate medium wicking from the liquid from the bottom of the tube up to the top of the strip (<5 min) prior to freezing at −80 °C

Results and discussion

Reactive latex biocatalytic coatings on hydrated paper substrate were prepared for all four organisms. This is the first report of a latex coating immobilization method for *C. reinhardtii* and *C. ljungdahlii* that retains cell reactivity as measured by oxygen gas evolution or CO consumption, respectively. Previous attempts to immobilize *C. reinhardtii* using latex binders with osmoprotective carbohydrates followed by drying at high relative humidity to promote polymer particle coalescence and adhesion resulted in no observable coating-associated activity, indicating that these microbes may be sensitive to desiccation stress. *Synechococcus*, *C. reinhardtii*, and *C. ljungdahlii* immobilized in latex coatings that were not dried prior to rehydration were reactive in the head-space of the Balch tubes. *Synechococcus* coatings and *C. reinhardtii* coatings consumed CO_2 (3.60 ± 0.20 and 3.90 ± 0.07 mmol CO_2 m^{-2} h^{-1}, respectively) and produced O_2 (5.00 ± 0.25 and 10.2 ± 0.2 mmol O_2 m^{-2} h^{-1}, respectively) when incubated in the head-space (Fig. 2a, b). *C. ljungdahlii* coatings, when incubated in the head-space of the microbioreactor, consumed CO and H_2 (6 ± 1 mmol CO m^{-2} h^{-1} and 1.84 ± 0.08 mmol H_2 m^{-2} h^{-1}) while producing CO_2 (2.17 ± 0.09 mmol m^{-2} h^{-1}); acetate and ethanol accumulated in the liquid phase at the bottom of the tube (Fig. 2c, d). The reactivity of hydrated *R. palustris* coatings was significantly reduced when incubated in the head-space of the Balch tubes (0.47 ± 0.04 mmol H_2 m^{-2} h^{-1}) compared with those submerged immediately after coating in

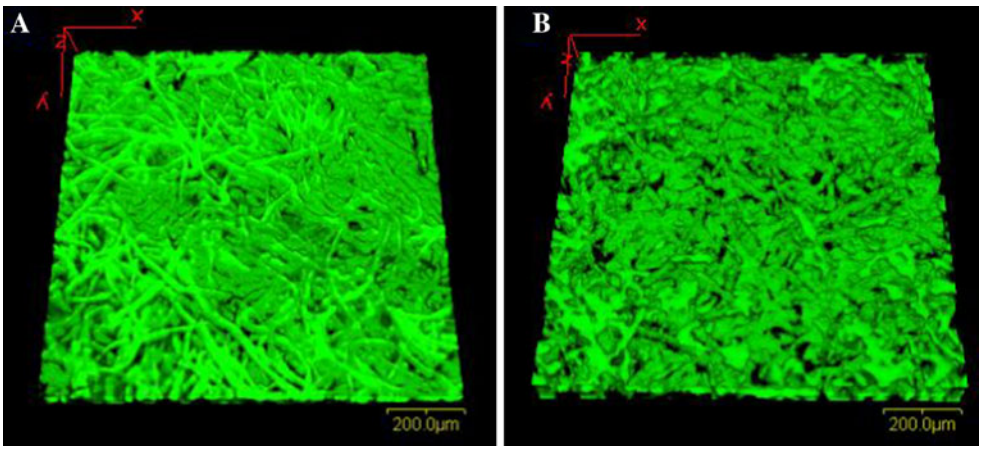


Fig. 4 Confocal laser scanning microscopy of deconvoluted *z*-plane tilted images of latex coated paper. **a** 3MM chromatography paper hydrated in BG-11 medium and **b** 3MM chromatography paper coated with *Synechococcus* PCC 7002 in SF012 latex binder emulsion and hydrated in BG-11 medium. Color is natural fluorescence of cellulose fibers; 512^2 pixels/1 mm^2 area

acetate-containing medium (5.8 ± 0.5 mmol H_2 m^{-2} h^{-1}). When the head-space-incubated *R. palustris* coatings were submerged in acetate-containing medium, hydrogen production increased (1.4 ± 0.3 mmol H_2 m^{-2} h^{-1}), indicating that poor acetate diffusion through either the porous paper or latex nanopores may have limited hydrogen production. This also indicates that oxygen production and carbon dioxide fixation observed for *C. reinhardtii* are independent of acetate present in the TAP medium.

The 3MM chromatography paper was 311 ± 13 µm thick and 322 ± 16 µm when hydrated and measured frozen using the digital micrometer and profilometer methods, respectively (Fig. 3). Due to the limitations of the manual pipette tip coating method, the porosity and surface roughness of the dry paper substrate, and the small patch surface area, the latex patch coatings were not uniform in thickness across the coated area, resulting in large standard errors in thickness measurements when using a digital micrometer. Frozen *R. palustris* coatings were 18–31 ± 2.0 µm thick on dry paper, and frozen *C. ljungdahlii* coatings were 7–15 ± 2.6 µm thick on dry paper; both coatings were thinner when the paper was fully hydrated. *Synechococcus* coatings were 35–58 ± 3.6–7.0 µm thick on dry paper, and the coating thickness was reduced when rehydrated. Because of the larger size of microalgae, *Chlamydomonas* coatings were 46–68 ± 3.1–5.3 µm thick on dry paper and thinner when rehydrated. The surface roughness of the frozen coated paper was determined by profilometry scanning from the coating center outward. The patch thickness varied by ±25 µm, with some outer edges having peaks of >50 µm due to the nonuniform manual coating method and the different sizes of the microorganisms. Coatings of *C. reinhardtii* had the greatest surface variability (data not shown). In spite of the limitations of coating uniformity using this manual deposition method, multiple coatings could be made rapidly, with sufficient cell density to measure gas-phase reactivity. In particular, using this latex coating method, all coatings were <75 µm thick, significantly thinner than the 200–500 µm thick *Calothrix* Ca^{2+}-alginate films recently reported to produce hydrogen gas [23].

Latex binders are commonly used in paper coating, where they partially fill the upper layers of the paper pores to form coatings from 10 to 30 µm thick. Visual evidence of 3MM chromatography paper porosity and latex paper coating thickness was obtained from *z*-plane deconvoluted CLSM images of *Synechococcus* PCC7002 SF012 latex coatings hydrated in BG11 medium (Fig. 4). The SF012 latex emulsion does not appear to significantly alter the open paper structure by plugging the pore spaces between the fibers (Fig. 4b).

The kinetics of wet coalescence of SF012 latex as a function of temperature and time has not been measured. However, the wet coalescence of a similar acrylate/vinyl acetate latex binder (Rovace™ SF-091) was previously measured by our group through the loss of effective diffusivity of delaminated films in a half-cell diffusion apparatus using a nitrate tracer. The kinetics of wet coalescence as a function of temperature from 5 to 30 °C was described by an Arrhenius relationship with activation energy of 108 kJ/mol [27]. This means that wet coalescence can be rapid depending on the polymer particle glass-transition temperature (T_g), surface chemistry, and incubation temperature. Determination of the kinetics of latex binder wet coalescence on paper, adhesion to cellulose fibers (or other nonwoven substrates), visualization of the microstructure of hydrated coatings, and accurate determination of the number of reactive microorganisms immobilized on the paper will all be important to further optimize this method. In addition, measurement and

control of the hydration film thickness on the surface of the coatings and characterization of the mass transfer of gases through this thin liquid film as resistance to transport into the coating need to be investigated further.

Cellulose fibers (saw dust, shavings, chips, etc.), cellulose-containing plant materials (peat), or compost are common microbe immobilization bioreactor supports for trickling bed bioreactors and biofilters for air pollution control [21]. Paper is used extensively as a substrate for biocomposite devices and for advanced disposable two- and three-dimensional analytical detection devices [9, 11, 15]. Many inexpensive latex polymer particle binders are formulated for good adhesion to cellulose when coalesced and remain adhesive when hydrated. This combination of an inexpensive open nonwoven cellulose fiber paper support and readily available nontoxic inexpensive latex binder currently used for high-speed paper coating may significantly reduce material costs for large surface area, supported membrane absorbers or bioreactors. In addition, the open pore structure observed following latex coating (Fig. 4b) not only increases gas–liquid–microbe mass transfer but also facilities separation of microbial metabolites (such as acetate and ethanol) secreted into the liquid-filled pore space which diffuse away from the cells. The open pore microstructure can also facilitate escape of gas bubbles (such as CO_2, H_2) from the biocatalytic coating.

The pore microstructure and mass transfer resistance of fluid-filled hydrated paper for supporting latex biocatalytic coatings differ from other types of membrane-supported bioreactors. Microporous membranes with liquid-impermeable layers at the gas–membrane interface are used to support natural biofilms on the bulk liquid side of the membrane, but this configuration has the disadvantages of uncontrolled reactivity due to biofilm outgrowth into the liquid phase and pore wetting on the gas-phase side [1]. Stacked or horizontal array membrane bioreactors have a hydrophilic membrane with pore size less than the microorganism size and are used to support growth of a natural biofilm or serve as a support for covalently attached or gel-immobilized biocatalysts covered by a thin liquid film in contact with the gas phase. This leads to extreme pressure drops if the membrane pores are plugged by biomass outgrowth. In contrast, the latex-coated paper method described herein relies on wet coalescence of the latex binder polymer particles to generate adhesion to the cellulose fibers without plugging of the paper pores. The partially coalesced latex particles generate nanoporosity surrounding the microorganisms (pores smaller than the microorganism), and the effectiveness of an additional coating of a thin nanoporous top coat of latex binder to further limit biomass release or nutrient limitation to prevent outgrowth has been demonstrated [12, 14]. In the method described herein, the hydrating liquid film thickness on the paper fiber coating surface in contact with the gas phase is supplied by the liquid-filled pores beneath the coating in equilibrium with vapor-phase water. The film thickness on the surface of the coating in contact with the gas can thus be controlled by the relative humidity. The mass transfer coefficient of gases in contact with the hydrated paper is directly proportional to the diffusion coefficient divided by the stagnant liquid film thickness. Therefore, generating data with a simple small-scale test system such as described herein where the liquid film thickness and loss of biocatalyst from the coating can both be controlled is a significant advantage for engineering and designing efficient large-scale microbial gas absorbers and biocatalytic membrane bioreactors.

The reactivity of this simple sealed tube system can easily be improved by increasing the coating surface area and cell density in the latex emulsion. When the coating strip is placed in the gas phase, both sides (coated and uncoated) are accessible to gas mass transfer through static liquid in the paper pores. However, due to the thickness of the chromatography paper, the mass transfer rate of gases dissolved in the static liquid in the pores from the uncoated side to the bottom of the latex coating is low, as the uncoated side does not contain cells. Gas consumption significantly increases the transfer rate of CO and CO_2 absorption in gas absorbers [5, 6, 26]. Thus, to increase the reactivity, both sides of the paper could be coated. One limitation of stagnant liquid-filled paper pores is that the products of microbial metabolism secreted from the cells accumulate in the fluid-filled pores and must diffuse tangentially down the paper into the bulk liquid at the bottom of the tube. This could be an advantage, as the paper support is acting both as a support and as a separation device. An additional approach to increase the reactivity of these systems is to promote liquid mass transfer through the paper pores tangential to the coating to minimize inhibition of the microbes in the coating by secreted products. It would be similar in this case to biocatalytic membrane reactors and analogous to falling film or "wetted wall" gas transfer devices described by film theory [6]. Open cross-flow catalyst supports are routinely used for reaction and separation in the chemical industry, and their engineering is well established.

Investigation of the microstructure of inexpensive paper or other nonwoven support materials that exhibit open pore structure following latex coating should lead to development of gas-phase biocatalyst supports capable of simultaneous product separation. These materials may be useful for reducing the accumulation of more toxic microbial products. In particular, we envision that this technology could enhance butanol production from CO, CO_2, and H_2 by strains of *C. ljungdahlii* transformed with genes encoding enzymes for butanol biosynthesis [22].

Conclusions

Although the simple gas-phase latex immobilization method described herein requires little more than cells, a nontoxic latex binder, stabilizing carbohydrates, and chromatography paper, the method has many potential applications. Reactive coatings can now be made using microorganisms that previously did not survive desiccation during latex film formation and drying. It is now possible to determine the molecular mechanisms of preservation of cell viability and reactivity as a function of drying stress, similar to a latex film formation toxicity assay [14], thereby giving a direct means of evaluating how the coating drying process influences cellular processes that affect the rate of biocatalysis. This method should be useful for screening for viability following coating and the effect of water activity, and in combination with transcriptome or proteome methods may facilitate new cellular engineering approaches to minimize loss of reactivity under the physiological stresses which occur during latex film formation and partial drying. In addition, the reactivity as a function of the mechanism of latex toxicity can be quickly determined for different types of microbes and latex polymer emulsion chemistries.

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