PowerCell Payload on Eu:CROPIS - Measuring Synthetic Biology in Space

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ABSTRACT

NASA's PowerCell payload, as part of the German Space Agency's (DLR's) Eu:CROPIS (*Euglena* Combined Regenerative Organic-food Production In Space) mission, will compare the effect of multiple simulated gravity regimes on basic processes required for synthetic biology in space including growth, protein production, and genetic transformation of the bacterium *Bacillus subtilis*. In addition, it will pioneer the use of a cyanobacterially-produced feedstock for microbial growth in space, a concept we call "PowerCell." The PowerCell experiment system will be integrated on the DLR's compact satellite as a secondary payload to be launched during the summer of 2017. In order to simulate the gravitational range of different celestial bodies, the satellite will establish an artificial gravity level in the 1.4% – 52% of terrestrial gravity range prior to conducting each set of biological experiments, with experimental results compared to ground controls. Experiments will be carried out in microfluidics cards with experimental progress measured through absorbance as detected by the LED-based optical system. Here we describe the ground studies that led to these experiments, along with a description of the experiment system hardware and its performance. The mission results will provide foundational data for the use and production of genetically engineered organisms for extraterrestrial missions.

INTRODUCTION

The use of microbes for resource production and recycling in space has the potential to reduce launch costs, enable long-term human exploration, and provide new mission capabilities. Past analyses have suggested that the use of available genetically engineered organisms could reduce food weight by 38% and manufacturing material by 85% with an associated increase in mission longevity[1]. Mission capabilities can be increased by producing vitamins, medicine, and

other substances vulnerable to degradation by ionizing radiation in the gulf of space[2], while structural materials can be produced in situ[3].

Yet, many questions remain unanswered with regard to biological behavior in micro- or reduced-gravity. Gravity plays a key role in cellular activity, providing hydrostatic pressure and compression, enabling thermal convection and mixing, and establishing density gradients for orientation within cells[4]. We know that cellular processes function abnormally under microgravity: low hydrostatic pressure can disrupt the cytoskeletal network[5], gene activation rates increase or decrease from a lack of mechanical stress, and developmental pathways are disrupted by the lack of pressure or density-dependent signaling[6].

While the *Euglena* Combined Regenerative Organic-food Production In Space (Eu:CROPIS) mission's primary payload will investigate gravitational effects on tomato plants, we will investigate if basic biological processes critical for a microbiology/synthetic biology-enabled mission will change in response to the gravitational force over a range that spans two probable future human destinations: the moon and Mars. If synthetic biology is to become an enabling technology for space exploration, we must understand how frequently-used chassis organisms such as *Bacillus subtilis*, and operations such as genetic transformation and exogenous protein production respond to gravitational stresses.

POWERCELL MISSION CONCEPT

The Original PowerCell

The PowerCell experiment began with a concept developed by the Brown-Stanford 2011 International Genetically Engineered Machine (iGEM) team[7]: What if we could co-culture photosynthetic microbes to produce nutrients to feed other cells naturally productive or bioengineered for specific tasks such as chemical, material or food production for use off planet? Cyanobacteria are photosynthetic (converting CO_2 to sugars) and many are diazotrophic (converting atmospheric N_2 into biologically usable forms of nitrogen). However, they are difficult to engineer. The solution was to make a cyanobacterial strain excel at producing and secreting extra photosynthate, allowing it to feed a second organism that is more easily modified to produce a range of products for use in space or on non-terrestrial bodies. For this purpose, *Anabaena spp*. 7120 was engineered to continuously secrete sucrose into its environment, resulting in the development of the *Anabaena* PCS1, or "PowerCell", strain during the course of the summer 2011 session of iGEM. For a flight production organism, *Bacillus subtilis* 168 and similar strains are ideal candidates, possessing flight heritage[8], exceptional hardiness[9], and a well-cataloged history of genetic modification[10]. The 2013 Stanford-Brown iGEM team prototyped a protein-based sucrose sensor for *B. subtilis* that indicated that PowerCell worked by producing a fluorescent protein when *B. subtilis* metabolized sucrose[11]. From the iGEM "proof-of-principles" we developed a full-mission concept and began lab tests for payload development. Presented here is a description of the lab-based developmental work as we prepare for flight.

PowerCell Aboard Eu:CROPIS

We have created the PowerCell payload for integration into the German Space Agency's (DLR's) compact satellite program as part of the Eu:CROPIS mission, to be launched in the summer of 2017. This platform will be ideal to test another question raised in the original iGEM concept: "Will there be a significant change to synthetic biology operations due to gravity?" If we are to use synthetic biology in space, we must understand how variable gravitational forces affect the insertion of new genes through transformation and their subsequent function. The satellite will establish artificial gravity by rotating about its axis, providing payloads onboard with reduced gravity that includes the lunar-to-martian range. The PowerCell payload on the Eu:CROPIS mission will investigate how different artificial gravity levels affect bacterial cell growth, genetic transformation, and exogenous protein production. In this manner the PowerCell mission will take the first steps in transitioning lab-based synthetic biology into a space exploration tool at-destination while demonstrating the practicality of its hardware for small-sat applications.

POWERCELL HARDWARE

The PowerCell hardware is an improved version of hardware flown on PharmaSat[12,13,14], a previous biological payload designed and built by NASA Ames Research Center. The PharmaSat mission, launched May 19, 2009 from NASA's Wallops Flight Facility, was a successful 96 hour test of the effect of antifungals on yeast growth in microgravity.

The PowerCell hardware consists of two hermetically sealed enclosures (each ~21 x 29 x 8 cm and 4.3 kg) as depicted by the solid model in Figure 1, which will be integrated onto the compact satellite. Each enclosure contains two separate and identical payload modules with a 48-segment 3-color optical density or absorbance measurement system, grow light system, microfluidic system for nutrient delivery and waste flushing, plus thermal control and internal environmental sensing including temperature, pressure, humidity, and

acceleration. The fluidic card design allows for multiple experimental conditions at each gravity regime in each of the four rows of the card's 4x12 array of experiment wells through independent fluid delivery. Each of the twelve wells within each row could in principle be loaded with a different sample, although the scientific requirements for replication translate into two to three experimental parameters per row. The optics system measures growth via LED light absorbance at several wavelengths and allows for photosynthetic growth.

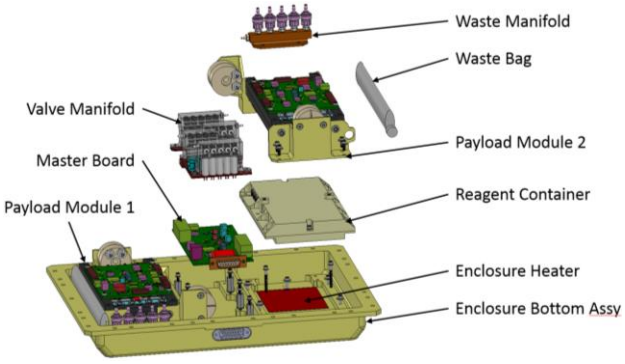

Figure 1 – Enclosure Layout

Fluidic Card

Each payload module has a self-contained fluidics system consisting of a reagent container assembly, a valve manifold for pumping fluids, the experiment card that receives the fluids, and a waste manifold/bag. The payload experiments are carried out within the experiment card. Each row is served by a separate fluid delivery line, allowing for four different fluidic protocols to be carried out within a single module. The wells are self-contained experimental samples, with 0.20 µm filters on the inlet and outlet to sterilize fluids and isolate organisms. The valve manifold uses four spring-and-solenoid valves for receiving reagents, and five magnetically latched solenoid valves for the outlets. The five outlets translate to each of the four rows of the experiment card plus an outlet to the waste manifold. The card is composed of sandwiched layers of poly(methylmethacrylate) ("acrylic") for the well sides with a clear gas-permeable 50-µm polystyrene capping layer to allow gas exchange. Fluidic cards were produced by ALine Inc (Rancho Dominguez, CA).

Optical Measurement System

The PowerCell payload measures optical density (OD) of the microfluidics wells at 3 wavelengths using an LED-based optical system. After rehydration with growth media, the *B. subtilis* grow within the sample wells and experiment progress is assessed by OD. As depicted in Figure 2, each well is aligned with its own AMS-TAOS light-to-frequency detector and set of three measurement LEDs with wavelength peaks at 430, 515 and 636 nm. The LEDs shine light through the well path and TAOS detectors measure the amount of transmitted light. Light is scattered (blocked) by cells (depicted by green and blue ovals) and the difference in transmittance over time used to measure growth. The 636 nm LEDs are useful for measuring cell growth, while comparison of 636 nm LED absorbance to the 515 nm and 430 nm LED absorbance can be used for measuring colorimetric assays. Although they are not part of our current experiment plan, the included broad-spectrum growth LEDs can also be used to facilitate the growth of cyanobacteria or other small photosynthetic organisms.

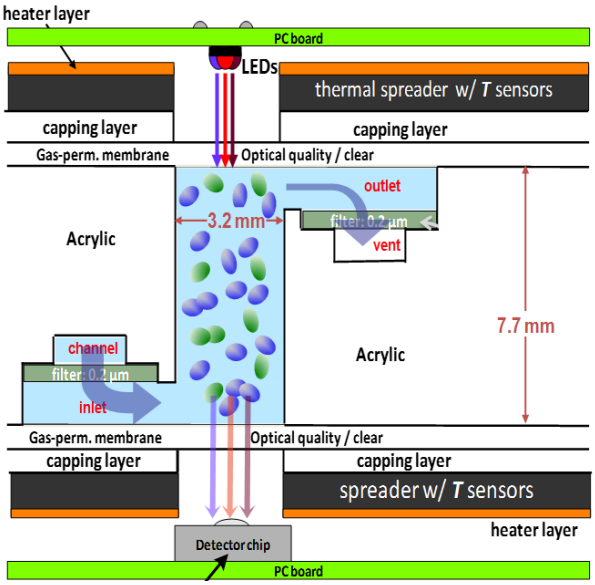

Figure 2 – Individual Well Cross Section

Electrical

The PowerCell payload electronics are based on those of PharmaSat[12,13,14]. Numerous improvements have been made to the design, and the newer, more miniaturized electronics used in the PowerCell payload allow for greater capability with a lower part count and smaller size. The electronics consist of a single Master printed circuit board (PCB) that interfaces to DLR's compact satellite bus and regulates power and communication, plus LED, Detector, and Valve Manifold PCBs for each experiment. To facilitate reuse on future missions, experiment electronics were designed to be compatible with a standard 2U small-satellite payload form factor supported by a 1U bus, or to interface with a master power/communications unit for use in a larger satellite as in the case of PowerCell's flight.

Software

The PowerCell system software was designed *de novo* with several advanced features built into the software architecture to allow significant configurability, fault tolerance, and the re-use of code for future biological payload systems. Rather than implementing a hard-coded experiment sequence in the software, a flexible onboard experiment "script" system was incorporated to allow arbitrary experiment sequences to be uploaded from the ground and then executed onboard. A complementary ground tool was developed for the generation of these experiment scripts. One of the advantages of this method is that as the desired experiment sequence was modified during the course of testing, the changes were isolated to the script and not the actual code – this dramatically reduced the amount of software modification during the test phase of the mission. There is also nothing that precludes the upload of a new experiment script during on-orbit operations in the event that a last-minute change is needed or desired.

Because the PowerCell electronics system largely consists of off-the-shelf components which are not radiation hardened, significant effort was made to mitigate single-event upsets in the software. Data redundancy, a multiple voting scheme, and memory scrubbing were some of the techniques used for this purpose. Other implemented features such as an onboard scheduler, bootloader, performance reporter, and a suite of complementary ground/operations software tools helped to increase the robustness of the system.

PAYLOAD SCIENCE EXPERIMENT OVERVIEW

Organism selection

Two microbes were considered for launch aboard PowerCell: the cyanobacterium *Anabaena spp*. 7120-PCS1 "PowerCell" and the common soil bacterium *Bacillus subtilis* 168. The ability to withstand long periods of dry storage drove organism selection, as launch constraints require the organisms to survive dark, dry, room-temperature storage for up to 2.5 years without careful temperature regulation prior to rehydration. Initial work focused on different methods of room-temperature stasis for these organisms. *Bacillus subtilis* has maintained 45% survival after one year of our simulated conditions. The *Anabaena spp*. 7120-PCS1 is the original concept organism genetically engineered by the 2011 Brown-Stanford iGEM team. *Anabaena spp*. 7120-PCS1 was dubbed "PowerCell" for the genetic modification that made it leak sucrose into its environment. Unfortunately, PCS1 is not naturally capable of forming the thick-walled dormant akinetes as other members of the genus do. Cells rarely survived longer than two weeks under the environmental constraints of the PowerCell payload. Because the requirement for long-term stasis was not being met, we were unable to fly living *Anabaena spp*. 7120-PCS1 on Eu:CROPIS.

Other organisms were considered for potential experiments, including *Escherichia coli* K12 as a production organism because of its widespread use in synthetic biology, and *Anabaena cylindrica* as a PowerCell as it does form akinetes. However, neither reliably survived beyond one month of storage during stasis testing, eliminating them from the final flight plans.

Cell Growth

Although numerous experiments have investigated the effects of microgravity on organism growth[4,8], there are no experiments to date on growth along a gravitational gradient below 1 Relative Centrifugal Force (RCF) as we intend to conduct. We will observe cell growth at 4 gravity regimes: 1.0 (on the ground), 0.52, 0.22, and 0.014 x RCF. These growth curves, which should provide information on low-gravity growth, will be conducted with *B. subtilis* strains 168 and 1A976 using a rich culture medium commonly used in lab experiments, Lysogeny Broth (LB)[15], and *Anabaena* extract as growth medium serving the role of PowerCell.

Genetic transformation

As synthetic biology operations rely on our ability to insert new genetic circuits and systems into production organisms, we need to assess the behavior of genetic transformation in destination gravity regimes for the use and maintenance of genetically engineered organisms during missions. Normally lab-based protocols would use electroporation, a method where an electric field is applied to cells to temporarily increase membrane permeability, or heat shock. Neither approach is feasible during the Eu:CROPIS mission. The transformation of chemically-competent cells requires multiple fast freeze-thaw steps and relies on fragile cells. With this in mind, we sought a simple method of transforming hardy cells within a standard production organism. For this we evaluated two isothermal one-step transformation methods for use in *B. subtilis* and *E. coli*. *B. subtilis* is naturally competent and adjustment of the procedure developed by Zhang and Zhang[16] with *B. subtilis* 1A976 led to a simple one-step process of transformation. For *E. coli*, polyethylene-glycol (PEG)-based transformation methods proved capable of transforming cells by mixing the cells and DNA with growth medium

containing 10% w/v PEG. While *E. coli* was not ultimately selected due to its poor performance during our stasis experiments, this result led to viable methods for future missions with similar limitations.

Protein Production

Since gene activation is known to change over time in space, a concern for synthetic biology is whether or not the activity of inserted gene(s) will also shift. By introducing an exogenous protein, β-glucuronidase, and two native promoters of characterized gene transcription strength, we will understand if the gene activation shift caused by lowered gravity significantly affects our ability to use unicellular organisms as production organisms as predicted by terrestrial lab-based experiments. We will examine the ability of *B. subtilis* to produce β-glucuronidase by growing cells in the presence of the X-gluc colorimetric assay[17]. The X-gluc assay functions when β-glucuronidase cleaves X-gluc ((5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid, cyclohexylammonium salt), forming a blue precipitate that our LED system can measure. We will compare the β-glucuronidase expression by the pVeg promoter – a highly expressed constitutive promoter – and the pLiaG promoter – a constitutive promoter repressed by cell stress. Table 1 lists the sequences of these promoters, both of which were obtained through the iGEM Registry of Standard Biological Parts[18,19]. We will determine if the mechanical stress shift across the simulated gravity range affects the native stress response of *B.* subtilis and, by extension, its ability to conduct exogenous protein production in extraterrestrial environments.

Table 1 – Promoters used in B-glucuronidase production

Promoter-BioBrick Part Number	Sequence
pVeg-BBa_K823003	'5-gagttctgagaattggtatgccttataagtccaattaacagttgaaaacctgcataggagagctatgcgggtttttattttacataatgatacataatttaccgaaacttgcggaacataattgaggaatcatagaatttgtcaaaataatttattgacaacgtcttattaacgttgatataatttaaattttatttgacaaaaatgggctcgtgttgtacaataaatgtagt-3'
pLiaG-BBa_K823000	'5-caaaaatcagaccagacaaaagcggcaaatgaataagcggaacggggaaggatttgcggtcaagtccttcccttccgcacgtatcaattcgcaagcttttcctttataatagaatgaatga-3'

METHODS – EXPERIMENT DEVELOPMENT

Spore Form Induction

B. subtilis spores were formed by nutrient starvation in Difco Sporulation Medium (DSM)[20]. DSM was made through the following steps: (1) Combine the following reagents/nutrients - Bacto Nutrient Broth 8 g, potassium chloride 1 g, magnesium sulfate heptahydrate 0.12 g, sodium hydroxide 0.06 g. (2) Add deionized water to 1 L. (3) Adjust pH to 7.5 with NaOH. (4) Autoclave and allow to cool to 50ºC. (5) Prior to use, add 1 mL of each of the following filter-sterilized solutions: 1 M $Ca(NO_3)_2$, 0.01 M $MnCl_2$, 0.001 M $FeSO_4$. Cells were incubated overnight at 37 ºC in 25 mL of sterile DSM. Cells from the culture were then diluted 1:100 into 500 mL of DSM with erythromycin (2 μg/mL) added and incubated at 37 ºC for two days. Samples of the culture were then observed with phase-contrast microscopy for spore formation. Incubation continued until >90% of observed cells were in the spore form. Once this condition was met, the culture was divided into 50 mL centrifuge tubes and washed twice with 70% ethanol by spinning at $6x10^3$ RCF for 1 hour, removing the supernatant, and re-suspending in an equal volume of filter-sterilized 70/30 ethanol/deionized H_2O. Tubes were then spun down a final time at the same RCF, the supernatant was removed and the cells re-suspended in 5 mL of 70% ethanol.

B. subtilis *Storage/Stasis Test Data*

To test that our *B. subtilis* spores would remain viable after dry storage in the fluidic card, we loaded 1 μL of *B. subtilis* 168 spore culture into each well of three fluidic cards and let them dry under a laminar flow hood overnight. These cards were then capped according to manufacturer's methods. A zero time point was taken by immediately filling one row of a loaded card with LB growth medium. Each well was then penetrated with a pipette and its contents re-suspended by mixing with the pipette. Ten μL of medium was then withdrawn from the well and serially diluted (10^0, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) then plated onto LB agar plates. Plates were stored at 37 ºC overnight, then inspected for colony counts to calculate the number of colony-forming units (CFU/mL). After plating, cards were immediately stored in hermetically sealed jars. At the time points of 1, 7, 30, 90, and 270 days, the above steps were repeated with a randomly selected row and CFU/mL calculated. The results are plotted in Figure 3. At 9 months of stasis we still see 45% ± 27% cell viability in *B. subtilis*.

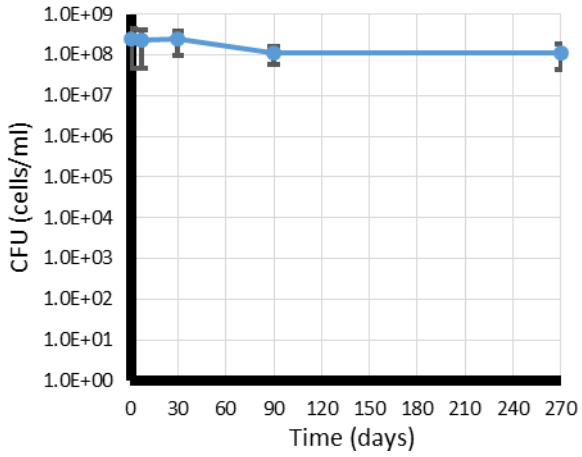

Figure 3 – *B. Subtilis* Cell Survival Counts after 270 Days

Lysate Extract production

The lysate nutrient mixture is derived from lysed concentrated *Anabaena spp.* 7210-PCS1 cultures. Dense PCS1 cultures were inoculated 1:100 into BG-11 freshwater medium[21] and grown to an OD_{600} of 1.0, then concentrated 1:50 through centrifugation at $2.2x10^3$ RCF followed by aspiration of supernatant. The concentrate was divided into 2 mL aliquots and sonicated for two minutes each using a Misonix Ultrasonic Processor XL with an amplitude of 2.5, pulsed at two seconds on-two seconds off. The aliquots were then centrifuged at $14.3x10^3$ RCF for one hour and the supernatant transferred to fresh centrifuge tubes. Aliquots were centrifuged at $14.3x10^3$ RCF for an additional 2 hours and the resulting supernatant transferred into sterile 50 mL tubes. The supernatant was then filter sterilized and ready for use.

Protein Production – GusA Reporter

The pMLK83 insertion vector, obtained from the Bacillus Genetic Stock Center, inserts the neo gene for neomycin antibiotic resistance and the gusA gene for β-glucuronidase production[22]. Two promoters of different strength – pLiaG and pVeg, described in Table 1 – were inserted into the pMLK83 vector at the 3' end of the gusA gene using the Gibson Assembly method (Gibson 2009). After successful insertion they were transformed into *B. subtilis* 1A976 along with un-modified pMLK83, referred to here as NeoR. Successfully transformed cells were isolated and cultured into spore stocks. Each spore stock's response to the X-gluc assay was measured in the following manner: three groups of four wells in a 96 well plate received 1 µL of spore stock for a total of twelve wells. Each group received 100 µL of LB growth medium and Neomycin (20 µg/mL) with a different concentration of X-gluc (0.6, 6, and 60 µL X-gluc/mL) added to each group. The plate was then placed in a SpectraMax spectrophotometer for incubation at 30 ºC for 65 hours. Absorbance measurements were taken every 15 minutes at 636, 515, and 430 nm, and a full absorbance spectrum was recorded at 24 hours. Figure 4 illustrates how the absorbance spectrum changes in cells with the pVeg promoter after 24 hours of incubation.

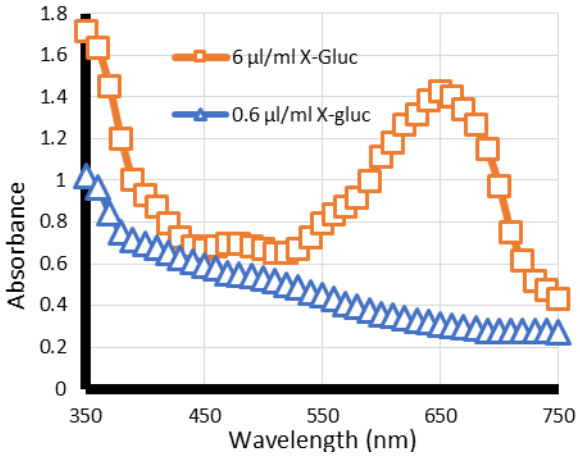

Figure 4 – Absorbance Spectra of pVeg Promotor Cells with X-Gluc Assay after 24 Hours.

Comparison of these spectra reveals a pronounced difference in absorbance at the spectral peaks of the green (515 nm) and red (636 nm) LEDs. We then use the ratio of 636 nm to 515 nm absorbance to measure the amount of blue precipitate produced relative to cell density and so compare the relative strengths of the promoters on Earth and in different gravity regimes. Figure 5 plots the result of this comparison at the 6 µL X-gluc/mL concentration. The stronger pVeg promoter cells clearly show a higher absorbance ratio compared to the weaker pLiaG promoter cells and the NeoR negative control.

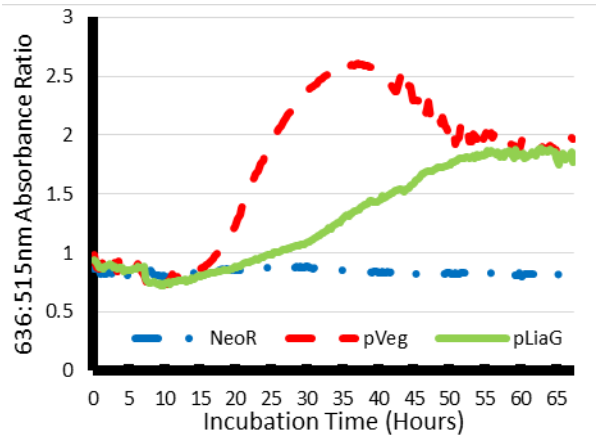

Figure 5 – Promoter Strength Evaluation Using 636 nm:515 nm Ratio with 6 µL X-gluc/mL

Supercompetent B. subtilis *Transformation*

B. subtilis strain 1A976 was obtained from the Bacillus Genetic Stock Center[23]. This strain is a genetically modified version of *B. subtilis* strain SCK6, developed by Zhang and Zhang[16], in which a xylose promoter was inserted to the 3' end of the competence master regulator gene ComK. Adding xylose to the growth medium increases competency as the xylose promoter increases the production of the comK, up-regulating DNA uptake and incorporation. In the original protocol, *B. subtilis* 1A976 is inoculated into 3 mL of LB medium with 1 µg/mL erythromycin and grown overnight at 37 ºC with shaking at 200 RPM. The culture is then diluted to reach an absorbance of 1.0 at 600 nm into a fresh LB medium containing 1% (w/v) xylose and grown for 2 hours. To transform, 1 µL of DNA is mixed with 100 µL of the cells in a test tube and incubated at 37 ºC for 90 minutes with shaking at 200 RPM. Cells are then serial diluted 10^{-3} to 10^{-4} and plated on LB agar plates with antibiotic for transformation selection. This method was optimized to meet the restrictions of the mission environment and work in the fully automated platform within our hardware.

Many different parameters were tested and optimized, including: (i) wet versus dry DNA and spores, (ii) different DNA protectants, (iii) spore concentration, (iv) ethylenediaminetetraacetic acid (EDTA) concentration in the TE buffer (10 mM Tris, 1% EDTA), (v) temperature range (23 ºC to 37 ºC), (vi) circular DNA versus linear DNA, (vii) mix versus no-mix, (viii) DNA concentration, (ix) neomycin timing test, (x) neomycin minimum inhibitory concentration (MIC) determination. Since the DNA concentration far exceeds the spore concentration, the transformation efficiency is essentially related to the initial concentration of spores. The concentration of spores is in turn limited by the pumping capacity of the fluidic system of hardware. To get the best out of these two parameters, we have determined that the best concentration is $3x10^6$ spores per sample well (70 µL).

In our experiments, linear DNA re-suspended in a modified TE buffer (10 mM Tris, 5% EDTA, pH 8.0) yields the highest transformation efficiency. The best pre-incubation time before neomycin addition was found to be 10 hours. The highest neomycin concentration yielding similar OD values between growth curves for a range of concentrations is 200 µg/mL, although 100 µg/mL is more similar to the no-antibiotic control. After the optimization process, we obtained transformation efficiencies approaching 1% in the laboratory setup under flight-like conditions.

EXPERIMENT TESTING IN PAYLOAD HARDWARE

"FlatSat"—functional but less than fully integrated prototypes of the fight payload—arrangements were used for the majority of experiment testing in hardware, with final verification occurring in a complete build of a PowerCell payload enclosure. The primary differences between FlatSat tests and full flight builds were that the FlatSats did not attach the hardware to the enclosure base and did not encase the fluidic bags in the reagent container assembly. Fluidic path lengths and hardware were otherwise identical.

Optical System Calibration

Calibration was carried out to convert the TAOS sensors' photon count frequency readings into equivalent absorbance measurements. Absorbance A is defined as

$$A = \log\left(\frac{I_o}{I}\right) \qquad 1$$

Where I is the intensity of light (number of photons/(time-area)), and I_0 is the initial light intensity in the absence of cells or other absorbing substances.

The detector board's TAOS sensors collect light and output frequency values linearly proportional to intensity, based on a factor corresponding to the wavelength of the light. Effectively:

$$I = a_\lambda f\ , \qquad 2$$

where f is the frequency, and a_λ is a conversion constant based on wavelength. This gives us:

$$A = \log\left(\frac{a_\lambda f_o}{a_\lambda f}\right) = \log\left(\frac{f_0}{f}\right)\ . \qquad 3$$

The experiment system hardware reports the frequency f, while the wavelength constants cancel out. From measurements with a calibrated spectrometer (Spectramax, Molecular Devices) we can obtain the absorbance A at frequencies relevant to our measurement LEDs. This allows us to calculate the constant f_0 using a rearrangement of equation 3:

$$f_0 = f \times 10^A\ . \qquad 4$$

We calculated this value f_0 for each well's LED/detector pair, and established that the values for an individual well location vary within a 7% net signal deviation between both individual cards and different LED/detector board assemblies. After establishing a reference f_0 value for each well location, this value will be used for absorbance measurements of payload samples in flight.

Stray light was analyzed as a potential factor that might skew the f_0 reference values. Amido Black dye was loaded into a fluidic card to block the main optical path, then light transmittance from all LEDs through the card to the detector was measured. The signal was found to be below the detection limit of the TAOS detectors. Based on this, it was initially disregarded as a factor. Then, three reference fluids of known absorbance were created using common food dyes and measured at the peaks of the LED wavelengths (636 nm, 515 nm, and 430 nm). The frequency f with de-ionized water and the three reference liquids in the fluidic wells was measured using the TAOS detectors in multiple cards and multiple hardware setups. The initial light intensity/frequency f_0 for each reference fluid was then calculated based on the known absorbance value.

The value f_0 was assumed to be unique to the well position as the deviation between assemblies at the same well position was lower than between well positions within an assembly. So the f_0 value calculated for a given reference dye was compared between identical well positions across our hardware setups. The standard deviation of f_0 values for a given well position was then calculated as a percentage of the f_0 value. Taking the average of this percent standard deviation, we see 5-7% average variance in signal across all hardware, card, and reference combinations. This was determined as within acceptable bounds for biological measurements.

Growth Controls

For growth curve testing, *B. subtilis* 168 and 1A976 spores cells were dried into fluidic card wells in one µL aliquots from 10^9 CFU/mL spore stocks in 70% ethanol. Growth medium was then pumped through the card and growth was measured using the optical system. Figure 6 shows the plotted results for growth in LB with the *B. subtilis* 168 and 1A976 strains grown at 30 ºC in one row (five wells each, two blanks) of a fully assembled enclosure. The plot describes absorbance of the red LED wavelength (636 nm) over time, starting at cell rehydration. As expected within a small volume, the cells rapidly grow under immediately available nutrients, then switch metabolism to grow slowly using different nutrients sources (diauxic growth).

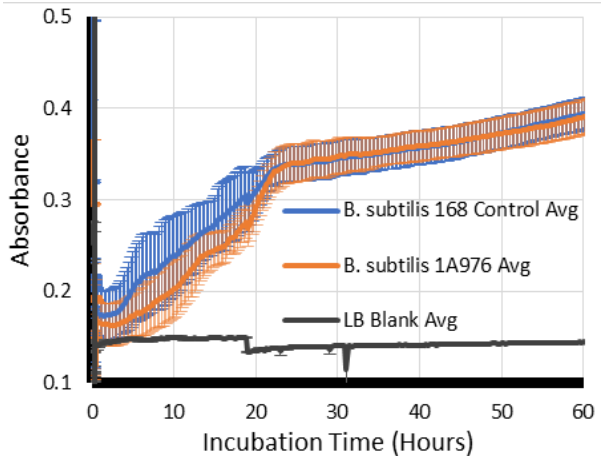

Figure 6 - Growth with LB Controls in Payload Hardware, Red LED Average Absorbance.

Transformation

For transformation testing, *B. subtilis* 1A976 spores were dried into eight wells of a fluidic card row in 1 µL aliquots from 10^9 CFU/mL spore stocks in 70% ethanol. In the same row, two wells were left empty (labeled "blank"). Two wells were filled with positive controls consisting of already transformed *B. subtilis* (1A976-NeoR) spores of otherwise identical concentration and volume to the experimental wells (+ control) while three wells consist of all elements of the transformation well without plasmid DNA (-control). Following cell drying, one µL of 40 ng/µL pMLK83 DNA in TE Buffer was dried in five of the 1A976 spore wells on the well walls opposite the spores. Figure 7 illustrates how this is arranged in the card. For hardware testing, three identical rows in this arrangement were created to test the incubation time needed for transformation.

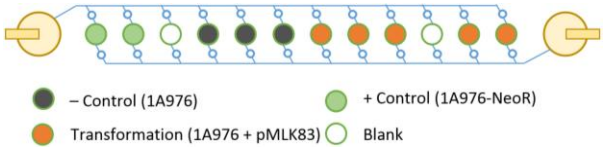

Figure 7 – Transformation Row Layout

The fluidic card was assembled in the FlatSat configuration, then rehydrated with LB+2% xylose. The rows were then flushed with LB+ Neomycin (100 µg/mL) after incubation at 30 ºC for 8, 10, and 12 hours. Transformation was assumed to be successful if absorbance increased above negative control levels to indicate growth in the antibiotic medium. As Figure 8 shows, excessive incubation allows the non-transformed cells to outcompete the transformed cells for resources, greatly delaying their growth post-antibiotic flush.

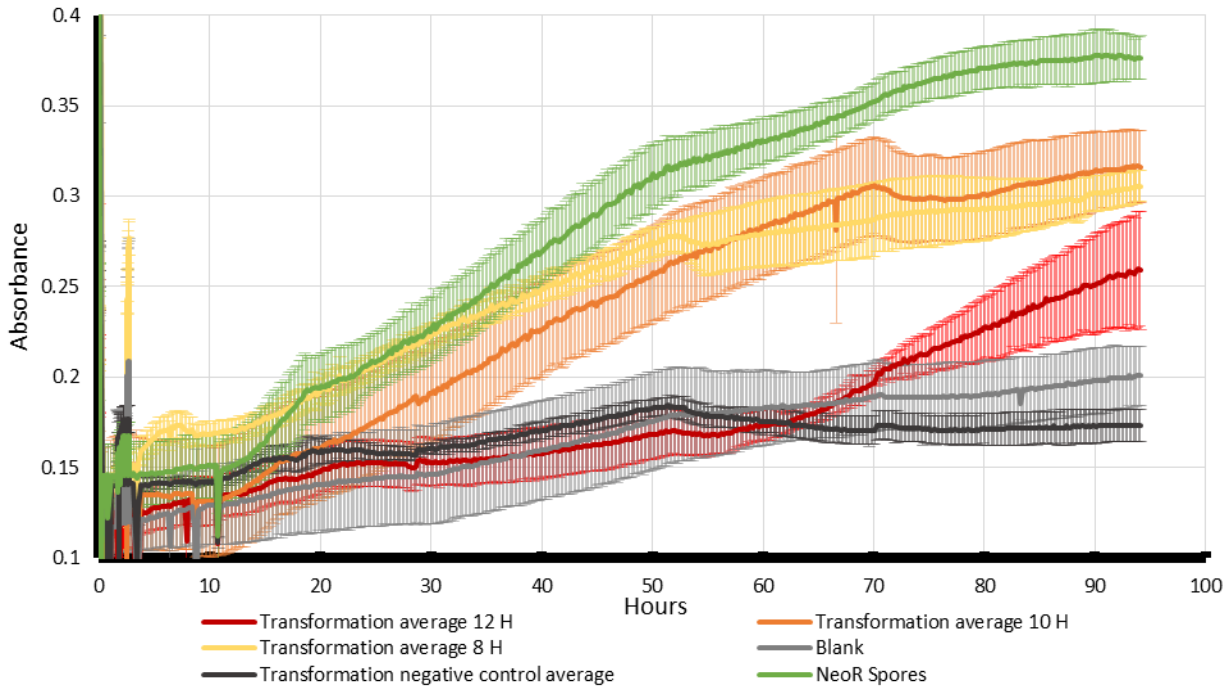

Figure 8 – Transformation Comparison in Payload Hardware: 8, 10, 12 Hour Incubation, Five Samples Per Time Point.

Protein Production Assay

The experiment system hardware tests were based on those conducted on the spectrophotometer. *B. subtilis* 1A976 was transformed with the blank pMLK83 vector as well as pMLK83 with the pVeg and pLiaG promoters for the protein production assay. The spore stocks were dried into fluidic card wells, then rehydrated with LB and Neomycin (50 µg/mL) with 1.2 µL X-gluc/mL. Measurements were taken every 15 minutes. The protein production was evaluated by comparing the absorbance ratios of 515 nm and 636 nm light (green and red LEDs), as demonstrated in Figure 9. The plotted response is lower than the spectrometer studies due to the lower concentration tested, but the hardware is sensitive enough to distinguish the strengths of the two promoters and the negative control.

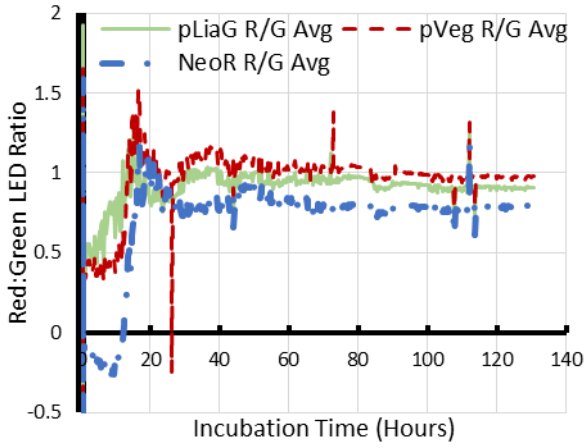

Figure 9 – B-glucuronidase Assay in Payload Hardware (Green:Red LED absorbance)

DISCUSSION

The PowerCell Payload hardware has the potential to be the starting point for an easy-to-use automated experimental system in satellite and other payloads. The microfluidic design allows for the sterile and independent addition of reagents, while the software's drag-and-drop scripting system allows for researchers with no programming experience to create automated experiments. The optical system has shown sensitivity

comparable to benchtop spectrometers and its LED wavelengths can be selected to accommodate different experimental conditions.

The PowerCell payload will address simple but important questions for the future of synthetic biology in space. By evaluating the role (variable) gravity plays in microbe viability and growth, it will inform us of the scope of applicable environments for genetically engineered bacteria to be used as an enabling technology. Understanding the subtle impact of gravity on the efficiency of characterized genetic parts like promoters can provide an approximation of how more complex genetic systems developed on Earth will respond to the stresses of new celestial bodies. Even confirming that genetic competency occurs to a degree similar to terrestrial operations allows better prediction of how genetic engineering operations might function in mission environments and be used as a dynamic tool for future development in non-terrestrial environments.

Synthetic biology has already shown enormous potential on Earth to create new medical technologies, materials, and fuels. The renewable nature of synthetic biology and life's own capacity for self-replication and resource utilization indicates great potential for its use in human space exploration. By translating these technologies to use in space, we are making the first steps to drastically reduce the cost and risk of manned space missions, especially on long-term operations. The first steps in testing its use are to re-create the conditions it might face in a mission environment and here we focus on the reduced gravitational force, bringing us closer to implementing synthetic biology as an enabling technology for space exploration.

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