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An Arduino based Automatic Pressure Evaluation System (A-APES) to quantify growth of non-model anaerobes in culture

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Abstract

Measuring the growth rate of non-model anaerobic microbes typically requires the use of time-consuming and often destructive manual measurements. Here, an Arduino based Automatic Pressure Evaluation System (A-APES) was developed to automatically measure the rate of fermentation gas production as a proxy for microbial growth in anaerobic systems. The A-APES system measures accumulated gas pressure in sealed cultures accurately at high-resolution, while venting the system at programmed intervals to prevent over pressurization. The utility of A-APES is demonstrated in this study by quantifying the growth rate and phases of a biomass-degrading anaerobic gut fungus, which cannot be otherwise measured via conventional techniques due to its association with particulate substrates. Given the utility of the A-APES approach, we provide a complete construction guide to fabricate the device, which is three times less expensive compared to existing commercial alternatives.

Keywords: Arduino; non-model microbe; anaerobic fungi; pressure transducer; automatic culture equipment

1. Introduction

Cultivation techniques applied to model microbes in biotechnology, like *Escherichia coli* and *Saccharomyces cerevisiae*, are well established, with many commercial tools available to automate data collection and analysis (Anand et al., 2019; Egli, 2015). Moreover, because model microbes are relatively simple to cultivate, and are well-suspended in batch or continuous culture, many lab-scale "do-it-yourself" devices have been constructed to facilitate high throughput, automated experiments that make use of optical density measurements and continuous recording of select metabolites (Bergenholm et al., 2019; Boccazzi et al., 2005; Groisman et al., 2005; Klein et al., 2013) to monitor microbial growth. However, non-model microbes often present unique difficulties that hamper direct application of these technologies and techniques, often necessitating time consuming and/or destructive manual measurements. For example, many such microbes have complex morphologies, are surface-adherent, and/or feature a complex life cycle (Podolsky et al., 2019).

Anaerobic gut fungi, in the phylum Neocallimastigomycota, are relatively understudied non-model organisms of high biotechnological value due to their vast array of carbohydrate active enzymes (Haitjema et al., 2017; Solomon et al., 2016; Youssef et al., 2013). However, anaerobic fungi have proven exceptionally difficult to characterize in large part due to challenges in their cultivation. They are strict anaerobes, temperature sensitive, filamentous and typically require specialized media for growth (Haitjema et al., 2014). Further, in contrast to model yeasts or fungi, anaerobic gut fungi are not well suited to cultivation in chemostats because they adhere to their growth substrates, and themselves, through a filamentous rhizoid network (Gruninger et al.,

2014). This necessitates either destructive harvesting of samples to benchmark cellular biomass or the use of indirect measurements to permit growth rate calculations.

Indirect measurements for anaerobes typically make use of accumulated pressure of fermentation products as a proxy for growth, and have been widely adopted in the field (Haitjema et al., 2014; Theodorou et al., 1995). For example, for anaerobic gut fungi, gas production rate growth curves are often used to study fungal lignocellulolytic properties and substrate preferences, yet are typically labor and time intensive to generate when fine resolution is required (Henske et al., 2018; O'Malley et al., 2012). Typically, the fermentation gas pressure in each sample under consideration must be measured and vented multiple times per day to obtain an accurate estimate of the fungal growth rate. The time intensive nature of measuring accumulated pressure in such cultures has led to the design and construction of devices that automate this process (Adesogan et al., 2005; Davies et al., 2000). In essence, these approaches typically combine a pressure transducer with a valve. The transducer measures the accumulated pressure over the course of growth, and the valve vents the closed system to prevent over-pressurization periodically, as shown schematically in Figure 1. Alternative designs include liquid displacement flow-meters, but accurate readings can be challenging to attain using such devices (Walker et al., 2009).

Despite the apparent simplicity of the design shown in Figure 1.A, these lab-built automated systems have not gained significant traction. This is likely because the electronics required to make these systems work are not simple or readily shareable. Relatively expensive commercial systems, such as the Ankom RF Gas Production System or the OxiTop Respirator system, exist and have been used to study the growth

characteristics of anaerobic systems (Pabón Pereira et al., 2012; Tagliapietra et al., 2010). On the other hand, Arduino based systems have recently become popular foundations to build lab automation devices of varying complexity (Urban, 2018, 2015). Importantly, Arduino based systems are low cost and relatively simple to build (Grinias et al., 2016; Sarik and Kymissis, 2010). There is also a growing drive to towards developing "open-hardware", which encompasses the development of low cost, easily shareable, standardized lab automation designs (Gibney, 2016; Sarik and Kymissis, 2010).

Here we use a non-model anaerobic gut fungus as a test bed to design and build a device that can be used to automatically record and release pressure to measure microbial growth. This enables the construction of high-quality growth curves for sensitive, strictly anaerobic microorganisms that are not amenable to direct biomass measurements. Specifically, this device measures and logs the rate of gas production and is particularly applicable to systems where the rate of gas production is correlated with biomass growth. The wireless Arduino based Automatic Pressure Evaluation System device introduced here, named A-APES, is specifically designed to work with strictly anaerobic systems, like rumen microbiome-based cultures. In particular, this system is designed to make use of standard lab equipment (serum bottles, incubators etc.) that are routinely used in the field. Use of this device will enable the collection of cross-lab comparable, high quality data without the need for significant manual oversight. Additionally, due to the use of the Arduino base and modular apparatus, it is straightforward to extend the system to include additional monitoring channels or simultaneously connect with other measurement devices if desired. The aim is to present a low cost, standardized system that can be built in any lab without the need to

understand complex electronics. We describe the design of the system, which includes a "ready to be manufactured" printed circuit board (PCB) that minimizes the amount of assembly and technical know-how required to construct the system.

Furthermore, to demonstrate the utility of the A-APES device, several high-resolution growth curves of an isolated anerobic gut fungus were constructed. Experiments were designed to investigate the influence of pressure venting frequency on the growth rate of anaerobic fungi. Additionally, these high-quality growth curves revealed that gut fungi appear to lack a true exponential phase when grown on lignocellulose. Instead, the growth rate appears to be multiphasic, possibly because the polymeric constituents of lignocellulose are not digested at the same rate by the gut fungus. The effect of venting frequency on the growth rate of the cultures was found not to be significant, suggesting that gas accumulation and venting frequency are not key drivers of the observed fungal growth rate. In future, the ability to accurately and continuously infer the growth rate of anaerobic gut fungi in real-time could be used to perform substrate optimization experiments for which current techniques are lacking in measurement frequency, sensitivity and precision.

2. Materials and Methods

2.1 Design and construction of A-APES

A schematic diagram of the Arduino based Automatic Pressures Evaluation System (A-APES) device is shown in Figure 2. The Supplement contains the Gerber file that was used to manufacture the printed circuit board (PCB), as well as other schematic documents that explain how to construct the entire device. Briefly, A-APES uses two XBEE ZIGBEE Mesh (DIGI, MI) devices for wireless communication between A-

APES and a computer that logs the data. The XBEEs are plug-and-play, requiring minimal setup through the free software XCTU from DIGI. The first XBEE is connected to the A-APES device; the second XBEE is connected to the data logging computer using an XBEE USB Dongle (WRL-11812, Sparkfun, CO). A short Python script is used to read and save the data from the USB connection (see the supplied code in the Supplement). Copper tubing, which is connected to an all metal syringe sealed with epoxy, is used to connect the solenoid valve (RSSM-2-12V, Electric Solenoid Valves, NY) and the pressure transducer (PX119-030AI, Omega Engineering, CT) to a bottle that is sealed using a 13 mm thick butyl rubber stopper typical for anaerobic experiments. Insulated 18-gauge wires are used to connect the solenoid valves to an independent power supply via a relay switch (Youngneer 5V relay, Amazon, WA). Additional wires (22-gauge) were used to connect the relay, which controls the solenoid valve, as well as the pressure transducer to an Arduino microcontroller (Arduino Uno R3, Amazon, WA) via the PCB, which used a second power supply. A 16-bit analog-to-digital converter (ADC) (1085, Adafruit, NY) is used to translate the transducer's output to a signal that is interpreted through the Arduino. More detailed information regarding the construction of the device may be found in Supplement (the construction guide, parts list and code).

2.2 Tubing and connections leak tests

Prior to the selection of copper tubing for A-APES, various other plastic tubing types were evaluated for their ability to form a gas tight seal between the pressure transducer, the needle and the solenoid valve, as depicted in Figure 2. This included Tygon (6516T11, McMaster-Carr, IL), Tygon PVC (8349T12, McMaster-Carr, IL), PFA (EW-06375-01, Cole-Palmer, IL) and CFlex (EW-06424-14, Cole-Palmer, IL) tubing.

To test the gas-tightness, each type of tubing was connected to a pressure transducer and left to equilibrate at 39°C in an incubator overnight. Subsequently, a 70 mL serum bottle, half filled with glass beads (2mm diameter, Chemglass, NJ), was pressurized to approximately 20 PSIa with pure CO_2 gas (representative of the typical operating conditions). This bottle was connected to the transducer and the pressure over time was monitored to ascertain the rate of gas leakage through the tubing. Copper tubing was used in the final design due to its superior gas tight seal, as is discussed later. The entire system was constructed, as shown in the Supplement, and leak tested. This entailed pressurizing three 70 mL serum bottles as before and recording the change in pressure over time.

2.3 Experimental evaluation of anaerobic growth

Standard anaerobic gut fungal culturing techniques and conditions were used for all the experiments presented in this work (Haitjema et al., 2014). All experiments used 70 mL (total volume) serum bottles with 0.5 grams of Corn Stover (supplied by the USDA-ARS Research Center, Madison, WI) in 40 mL of MC media (Davies et al., 1993), incubated at 39°C with a 100% CO_2 gas headspace. The filled serum bottles were autoclaved at 121°C for 20 minutes prior to use. An anaerobic gut fungus isolate, *Neocallimastix lanati*, was exclusively used in all the experiments. Each experimental triplicate was inoculated with 2 mL from the same 2-day old serum bottle of growing fungus of the same media composition as the experiment. Additionally, 0.5 mL of 10 mg/mL Chloramphenicol (BP904-100, Fisher Scientific, CA) was added to each bottle to prevent contamination by other microbes. Butyl rubber stoppers were used in all the experiments to ensure a gas tight seal between the serum bottle and the A-APES needle (as described above). Each experiment was run until stationary phase was observed,

typically 4-5 days post inoculation. Any deviations from this are noted in the relevant results section. Three independent pressure measurement (transducers) and release valves (solenoids) were used to enable the measurement of culture growth in a triplicate set of serum bottles. The venting frequency of headspace gas was varied as noted in the results section. Pressure measurements were taken every minute and recorded.

2.4 Data analysis

The experimental design resulted in three high resolution pressure measurement datasets per run. The growth rate for each dataset was determined by log transforming the cumulative pressure data and fitting a straight line to time-axis discretized intervals of 12 hours (approximately one doubling time) beginning 20 hours after inoculation. This yielded instantaneous growth rate data over the entire time course as shown in later figures. The 20-hour time offset was used to allow the system to equilibrate post-inoculation. For each replicate, the maximum straight-line slope over all the discretized intervals of the experiment was taken as the maximum growth rate of the dataset. Repeats of runs (each run is a triplicate set) were considered consistent with each other if the p-value of the unequal variance T-test was above 0.05 for over 50% of comparisons between the pressures measured at equivalent time points. The growth rates of different run conditions were also compared using the unequal variance T-test with a cutoff p-value of 0.05. The Julia language, as well as the packages documented in the code in the Supplement, were used for all the data analysis and visualization (Bezanson et al., 2017).

3. Results and discussion

3.1 A-APES is straightforward to construct and is gas tight

Here we introduce an Arduino based Automatic Pressure Evaluation System (A-APES) that can be used to automatically record and vent the pressure in anaerobic cultures. This system allows for the generation of high quality and high-resolution pressure accumulation data that can be used to infer the growth rate of non-model anaerobes in culture. A complete parts list and guide to constructing A-APES is shown in the Supplement. Due to the use of the Arduino base, minimal knowledge of electronics is required to build, modify and operate the system. Moreover, the PCB is designed to reduce the wiring and assembly time required to build the system, which is also relatively inexpensive compared to commercial alternatives. The cost to build the base system, i.e. A-APES with a single pressure measurement and venting unit, is approximately $430 (as of 2020). The cost for a fully equipped base system with 4 independent pressure measurement and venting units is approximately $1000. This equates to a price of $250 per measurement unit, which is 3.2 times cheaper per measurement unit than the equivalent cost of a commercial system. Beyond the cost savings of A-APES, the Arduino base makes the system readily extendible to include other sensors or configurations. Specifically, the high accuracy 16-bit ADC is not restricted to the pressure transducer. Therefore a wide range of commercially available environmental sensors with analogue outputs can also be monitored by the system, see (Urban, 2018) for examples.

Due to limited incubator space and media costs, it is also desirable to minimize the volume of culture vessels used with automated systems. To the best of our knowledge, the smallest operable working volume for a commercially available system is 250 mL. Filling a large bottle with a relatively small volume of liquid media results in a large headspace volume in the bottle. This larger headspace volume reduces the sensitivity

of the measured pressure in the bottle. On the other hand, using more liquid media relative to vessel size results in a smaller head space volume that can exacerbate the effect gas leaks have on the measured pressure. Thus, an important design requirement is that the measurement system is gas tight to accurately measure gas production rates, as well as maintain anaerobicity. A-APES is designed to be gas tight and not constrained to a particular bottle size. For demonstration purposes we used 70 mL total volume glass bottles filled with 40 mL of liquid media. However, it should be noted that the A-APES can potentially be used with a wide range of vessel sizes if they are sealable with butyl-rubber stoppers.

Various tubing types were considered and evaluated during the construction of A-APES, with the goal of identifying the most gas tight configuration. Figure S1 shows that plastic tubing leads to significantly higher gas leak rates, either due to the permeability of CO_2 and/or the barbed connection fittings that were used. Copper tubing was selected because the rate of gas leakage was the lowest (0.002 PSI/h), see Figure S1 for details. Since copper is not as flexible as plastic, some strain is placed on the connections when new serum bottles are connected to A-APES. This strain introduces the potential for leaks if the connections are not tight. Sealing the joints with epoxy solves this problem; it was found that the leak rate was halved in the final assembled system when epoxy was used to seal the joints, see Figure S2. However, using epoxy makes the connections permanent – a problem if the system needs to be disassembled and reconfigured. On balance the superior gas tightness ensured by the epoxy was deemed worth the inconvenience of permanent fixtures. The final gas leakage rate for the assembled system is 0.002 PSI/h. Assuming a 5-day run duration, and 25 PSI of

accumulated pressure (typical values recorded), leakage caused an error of less than 1% which we consider to be negligible.

3.2 No significant differences were observed between A-APES and manual pressure measurements of anaerobic fungal cultures

Pressure measurement differences between using A-APES and manually measuring and venting culture vessels were investigated by running a side-by-side comparison. It is important that the A-PES system is able to recapitulate pressure accumulation data measured manually because this is the standard in the field and would lend credence to novel observations derived from automatically generated data. To this end, A-APES was programmed to vent a set of triplicate anaerobic fungal cultures every 12 hours, while another set of triplicate cultures were started at the same time, from the same inoculum, and vented manually at the same interval. Figure 3.A shows the pressures at each measurement interval, and Figure 3.B shows the cumulative pressure profile. In both cases there were no statistically significant differences between the experiments at any point in time, as shown in Figure S3. Furthermore, the automatic experiment had a maximum growth rate of 0.087 ± 0.006 1/h, while the manual experiment had a maximum growth rate of 0.09 ± 0.012 1/h calculated by log transforming data points at the same time and finding the maximum slope for each experiment using these data points. The growth rates were also not statistically significantly different.

It is informative to note some differences between the manually and automatically vented cultures, which were enabled by this comparison. The manually vented cultures cooled down slightly during each measurement bout. While the effect of the temperature fluctuation on growth is likely small when measuring infrequently, it could

play a more significant role when smaller test tubes are used instead of individual serum bottles and/or measurements are done more frequently. Additionally, by removing the serum bottles from the incubator some stirring/mixing occurs. This is completely absent from the cultures that were measured using A-APES, as they are never removed, or moved at all, from the incubator. Despite these physical differences, the results suggest that A-APES measures growth rates and pressure profiles with no significant difference to the manual experiment, albeit with reduced manual labor.

3.3 A-APES demonstrates high run-to-run consistency

The reproducibility of A-APES was tested by comparing the pressure profiles and growth rates of two runs done at different times using the same venting frequency. Figure 4.A shows the measured spot pressures, and Figure 4.B shows the cumulative pressure profile over time for both sets of triplicate runs. The cumulative pressure profile is not significantly different over the entire growth curve, while the spot measurements are not significantly different over 89% of the growth curve, see Figure S4. Interestingly, the maximum growth rates were found to be statistically significantly different, irrespective of the time interval used to calculate, them as shown in Figure S5. The low measurement noise associated with the A-APES system likely makes any experimental or biological noise more noticeable, which gave rise to the significant differences noted in Figure S5.

The average difference between the maximum growth rates (as a function of different time discretization) was 0.01 ± 0.002 1/h. A leak test was performed to rule out that a leak in the connections caused the observed differences; this was found not to be the case. Thus, it is likely that these differences have a biological origin, as opposed to

indicating problems with A-APES. The inoculation method is the most likely explanation for the observed differences. Due to the sensitive anaerobic nature of the gut fungi, a set volume of a growing culture is injected into a serum bottle of fresh media to inoculate experiments. This makes controlling the precise starting biomass between runs challenging as batch-to-batch variability effects are difficult to minimize. Coupling the imprecise inoculations with the very precise pressure measurements likely led to the observed differences. Despite these observations, the high similarity in the measured pressure profiles suggest that A-APES is indeed consistent between runs. Furthermore, this result suggests that caution should be exercised when interpreting growth rate differences that are statistically significant yet small (on the order of 0.01 1/h) for this type of organism.

3.4 High resolution data yields accurate rate information over the entire growth curve

Manually measured pressure data is typically limited to very few data points, such as measuring and venting an anaerobic culture 3 times per day for 5 days, which results in 15 data points. On the other hand, A-APES can record measurements every minute, yielding much finer resolution that can capture significantly more growth dynamics (~15 vs. ~7200 data points, manual vs. A-APES respectively measured for 5 days). This allows for the inference of growth rates over the entire time course, with much higher resolution compared to manual methods. Figure 5 reveals that the growth rate of *N. lanati*, on a lignocellulosic substrate (corn stover), is variable. In particular, the growth rate seems to plateau for only a short duration (~5 hours), after which it decreases rapidly. By using the high-resolution data afforded by A-APES, it is apparent that classic exponential phase (characterized by a constant maximum growth rate) is absent.

Instead a multiphasic variable growth rate is observed. This information would be obscured by using lower-resolution manual methods. It is possible that the fermentable sugars released during the digestion of the lignocellulose by the fungus are differentially metabolized. This substrate preference could be the cause of the observed variable growth rate. In this case, harder-to-metabolize substrates are metabolized last, explaining why the growth rate starts to decrease midway through the time course.

Alternatively, it has been suggested that hydrogen production and accumulation inhibits the gut fungal energy metabolism (Gruninger et al., 2014; Marvin-Sikkema et al., 1994). To investigate this using A-APES, the venting frequency was varied (every 1, 4, and 12 hours in triplicate), and the growth rates were compared. By venting more frequently, the partial pressure of hydrogen would be reduced, differentially attenuating possible inhibition effects. However, as shown in Figure 6, it seems unlikely that this type of inhibition plays an important role in the observed growth rate decrease. Across all three conditions the growth rate profiles were similar and the observed maximum growth rates were approximately similar (~0.08 1/h, within the 0.01 1/h margin noted earlier). This suggests that pressure accumulation, and by extension hydrogen accumulation, does not significantly reduce the growth rate of *N. lanati*. While the reason for this observed growth rate decrease in anaerobic fungi remains unclear, the data suggest there is significant scope to experiment with conditions that optimize growth and to engineer anaerobic gut fungi to grow at their maximum rate for a longer time duration. In sum, the benefit of using A-APES is apparent here: very high-resolution data is available to interrogate the effect of experimental perturbations on sensitive anaerobic systems.

4. Conclusion

Here we have introduced a fully automated pressure measurement and venting device (A-APES) that can be used to infer the growth rate of microorganisms where gas production is related to biomass accumulation, such as anaerobic gut fungi (Haitjema et al., 2014). The device is also relatively simple to construct and operate. It affords the user high resolution gas production information that can be used to non-invasively study microorganism growth dynamics. Furthermore, due to the Arduino base the device is easy to extend and modify if desired, possibly paving the way for the construction of a lab-scale chemostat tailored for rumen-based microorganism systems. Additionally, we have used this device to reveal the growth dynamics of a non-model anerobic gut fungus. Due to the very high-resolution data afforded by the device, it is apparent that gut fungal growth is punctuated by a short regime of very rapid growth, followed by a much longer regime where the growth rate slows down. This suggests that the slow growth rate associated with anaerobic gut fungi may be heavily influenced by culturing techniques, rather than internal metabolic limitations.

5. Acknowledgements

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386 University entrepreneurial fund.

Figure 1

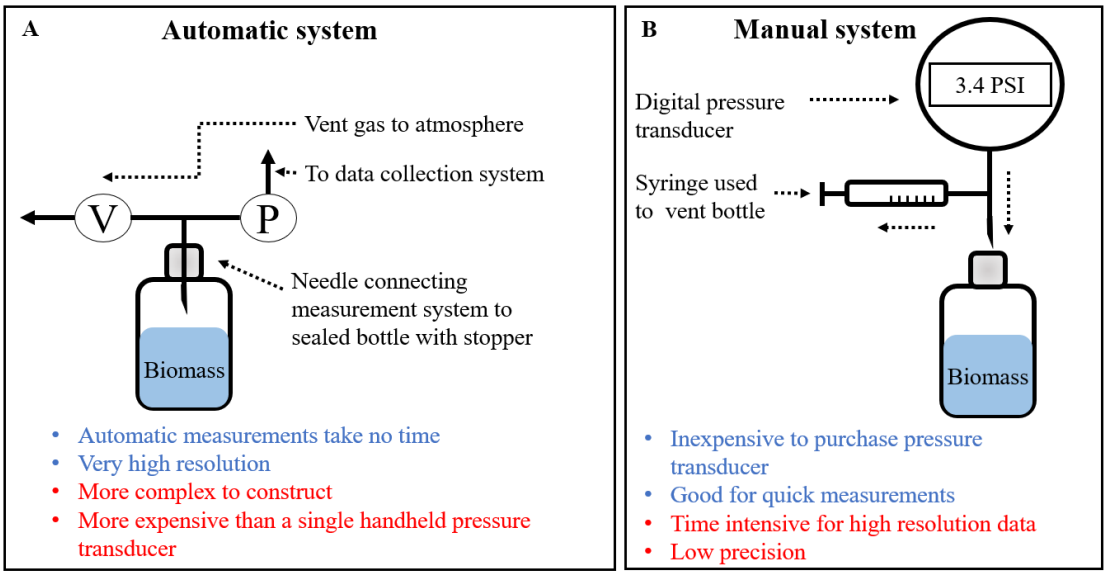

Figure 1: Conceptual design of automatic pressure measurement and venting devices (Davies et al., 2000) compared to labor intensive manual measurements. Benefits of each system are shown in blue font, with drawbacks in red. **(A)** Designs typically make use of a pressure transducer (P) that measures the rate of pressure increase in a sealed bottle, which is correlated to growth in rumen microbiome based systems (Haitjema et al., 2014; Theodorou et al., 1995). To prevent over-pressurization of the sealed bottles a valve (V) can be used to vent the system. **(B)** Manually measuring and venting the pressure requires the use of a handheld pressure transducer that is used to measure the pressure in the bottle prior to venting. Slight cooling of the bottles is usually observed due to the time it takes to vent the culture outside of an incubator.

Figure 2

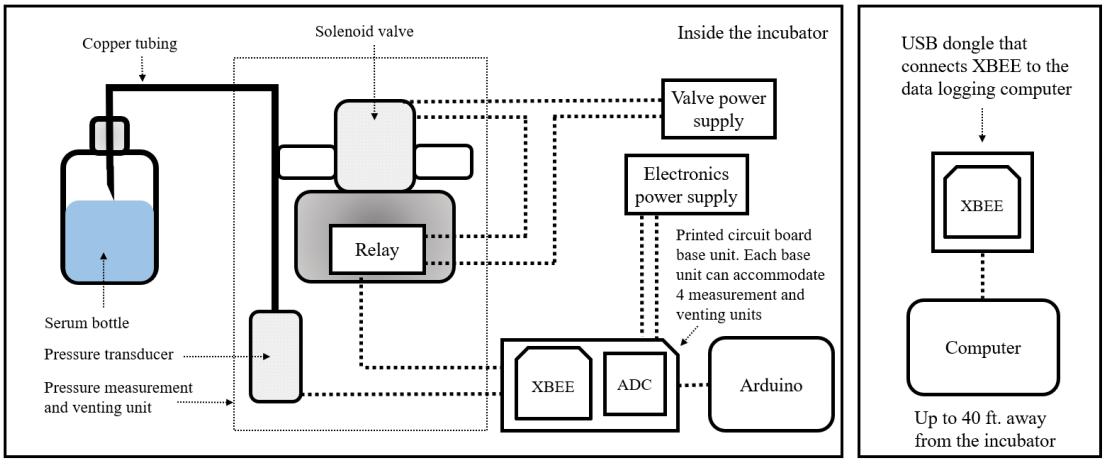

Figure 2: A schematic diagram of the primary components of A-APES. In this diagram only a single solenoid valve/pressure transducer unit is shown, but the base system can accommodate up to 4 independent units in total. The construction guide illustrates the assembly process (refer to Supplementary Information).

Figure 3

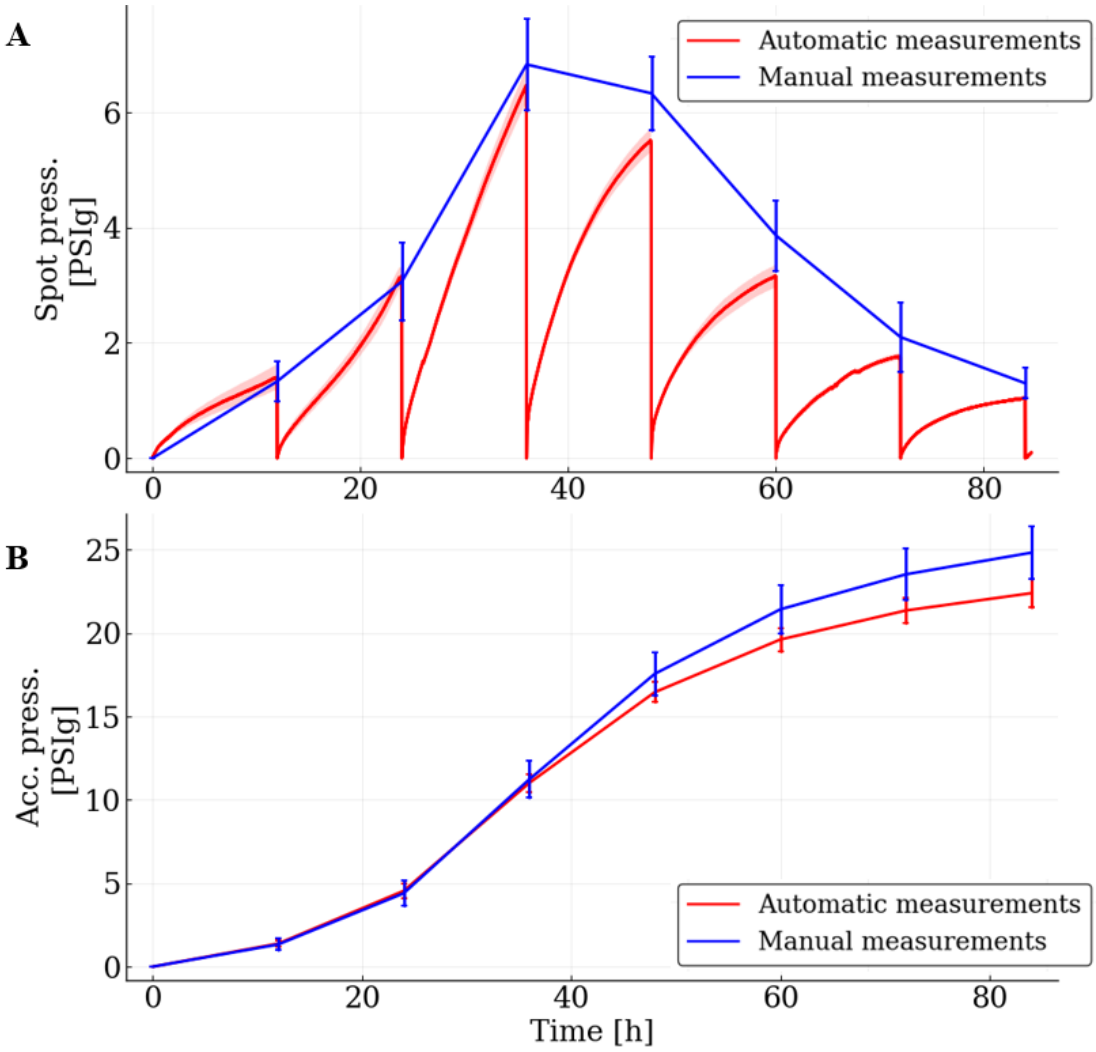

Figure 3: No statistically significant differences were found when comparing A-APES pressure measurements to manual pressure measurements of fungal growth. The pressure production measurements of two sets of triplicate *N. lanati* cultures were compared in a side-by-side experiment. Each replicate in both triplicate sets were treated in exactly the same way (2 mL inoculum from the same starter bottle into 40 mL complex media with 0.5 grams of corn stover, see the methods section for more details), except for the measurement method. One set used conventional manual pressure measurements and the other set used A-APES to record the pressure production rate. Both triplicate sets were vented every 12 hours. **(A)** Spot pressure measurements over time for both sets of triplicates. **(B)** The accumulated pressure

profiles for each case. Neither the spot pressure measurements (Figure 3.A), nor the accumulated pressure profile (Figure 3.B) was statistically different. The measurement noise was lower using the automatic system (shaded region in Figure 3.A represents 1 standard deviation). All error bars represent 1 standard deviation of error from the mean.

Figure 4

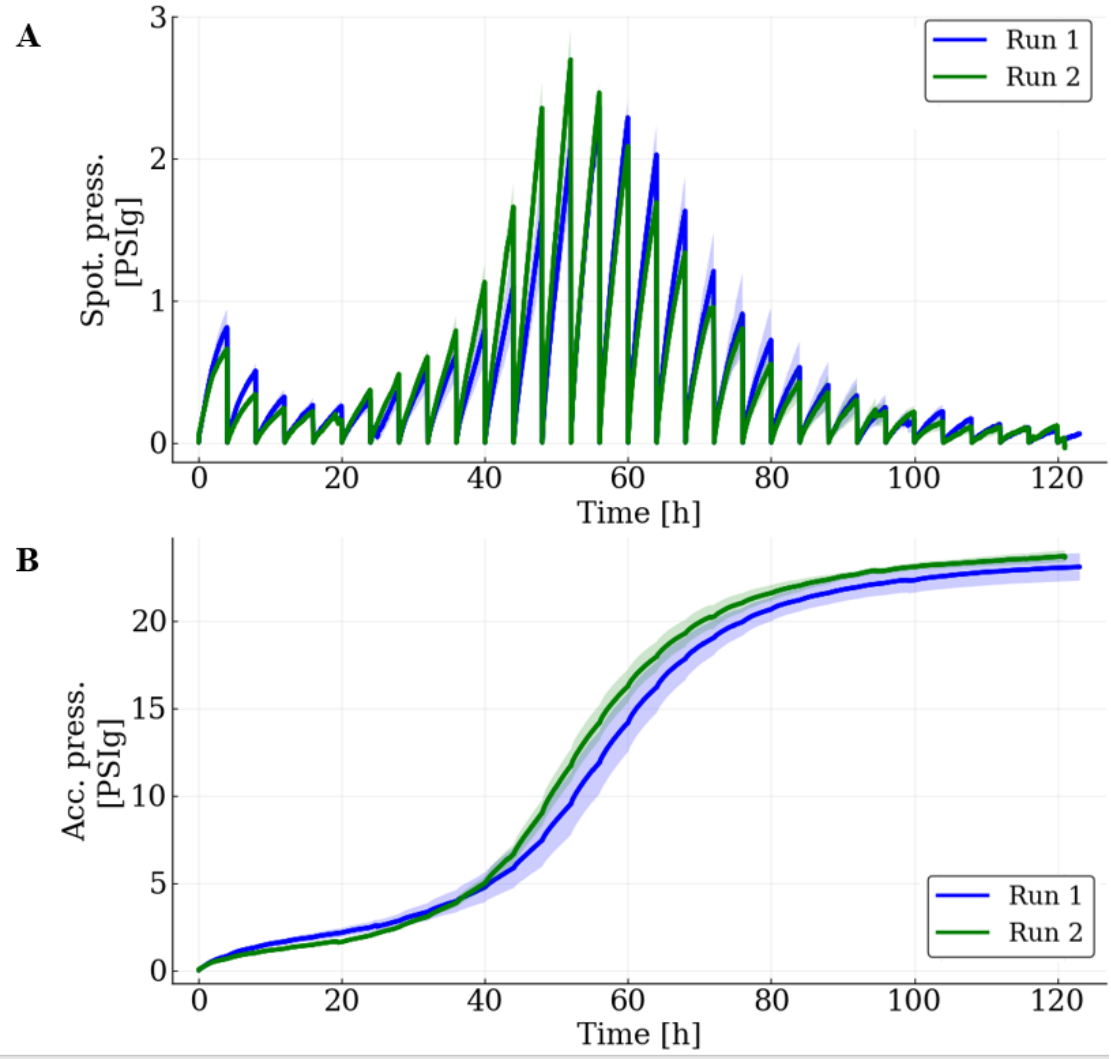

Figure 4: A-APES shows high run-to-run measurement consistency with minimal statistically significant differences. Two triplicate experiments (run 1 and run 2, respectively), using exactly the same experimental conditions (2 mL inoculation of *N. lanati*, 40 mL complex media with 0.5 grams of corn stover, venting every 4 hours and recording pressure measurements every minute, see methods section for more details), were run at different times to gauge the reproducibility of pressure measurements using A-APES. **(A)** The spot pressure measurements for each run. **(B)** The accumulated pressure profiles for each run. The shaded area represents 1 standard deviation from the mean curve. The spot pressure measurements (Figure 4.A) were not significantly

439 different over 89% of the experimental duration, while the accumulated pressure curves
440 (Figure 4.B) were not significantly different over the entire duration of the experiments.
441

Figure 5

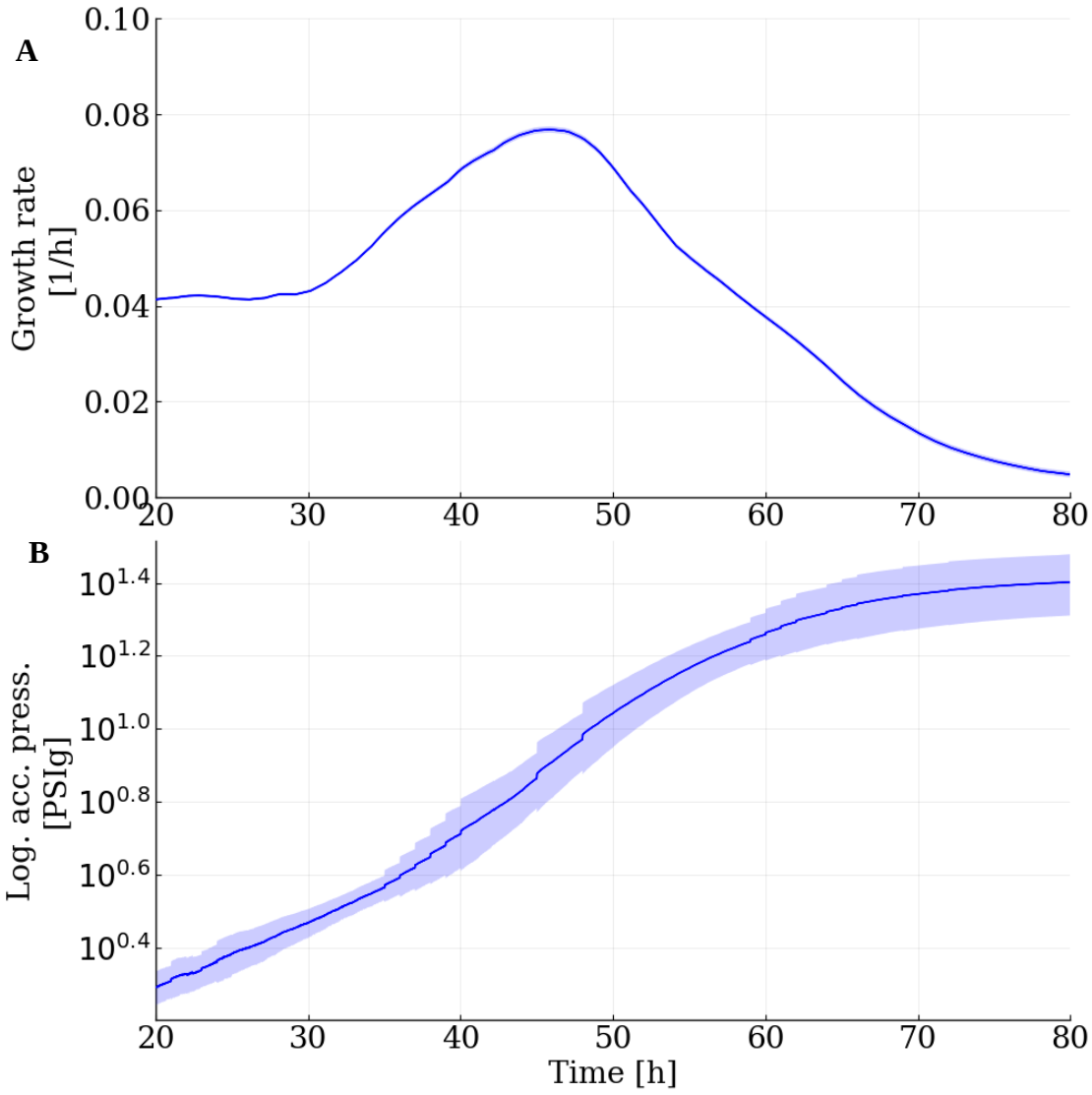

Figure 5: High resolution pressure measurements reveal that the growth rate of *N. lanati*, growing on a corn stover, is variable across the growth curve. Pressure was vented every hour, and measurements were taken every minute. Each replicate of the triplicate data shown here was grown in complex media with 0.5 grams of corn stover and inoculated with 2 ml from the same starter bottle, see the methods section for more details. **(A)** Figure 5.A. shows the inferred instantaneous growth rate, calculated over 12-hour intervals, peaks at ~0.08 1/h, but only for a short duration (~5 hours). **(B)** Figure 5.B. shows the corresponding log transformed accumulated pressure curve. In

455 both cases it is apparent that a classic constant rate exponential phase is absent.
456 Differential substrate digestion and metabolization may explain the variable growth
457 rates. For each figure the shaded region represents 1 standard deviation from the solid
458 blue curve that represents the mean of the measurements.

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Figure 6

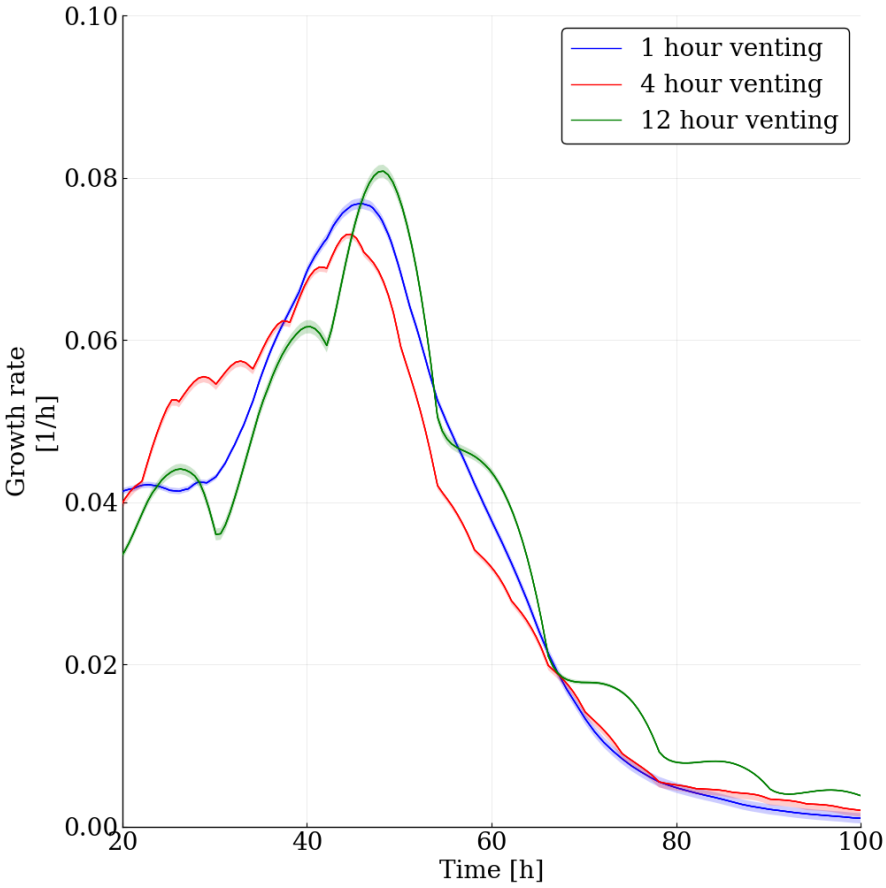

Figure 6: The observed instantaneous growth rate is not a function of the venting frequency, suggesting that pressure accumulation does not adversely affect the growth rate of *N. lanati*. Thus, it is unlikely that hydrogen inhibition plays an important role in the observed growth rate decrease. Three triplicate sets of *N. lanati* growing on 40 mL of complex media and 0.5 grams corn stover were vented at 1, 4 and 12-hour intervals to investigate the effect venting time has on the growth rate of the fungus. Higher venting frequencies reduces the buildup of pressure in the closed system, leading to lower concentrations of the gaseous fermentation products. The maximum spot pressure observed during the 1-hour venting experiment was 0.71 PSIg, suggesting that there was no significant buildup of hydrogen. In contrast, the maximum spot pressure

during the 12-hour venting experiment was 7.1 PSIg. In both cases the growth rates were comparable. The growth rates were calculated using 12-hour intervals, and the shaded region represents 1 standard deviation from the solid mean curve. Media de-gassing effects can be seen by the small “humps” in the 4-hour and 12-hour curves. The higher the venting frequency the more attenuated the de-gassing effect becomes.

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SI: An Arduino based Automatic Pressure Evaluation System (A-APES) to quantify growth of non-model anaerobes in culture

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Figure S1

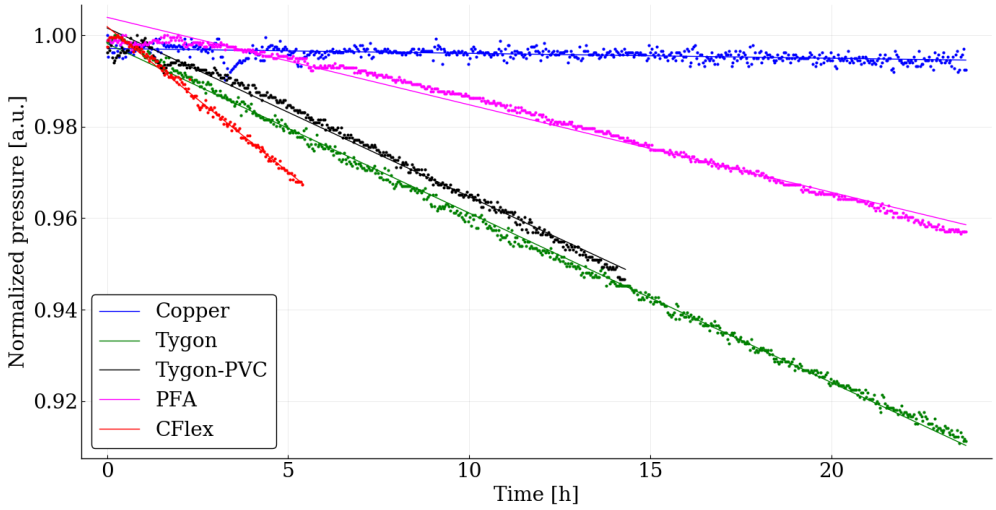

Figure S1: Normalized gas leakage rates for a variety of tubing types. The leak rate of various tubing types was tested by measuring the rate of pressure decrease in butyl-rubber stopper sealed 70 mL bottles half filled with 2 mm glass beads and pressurized to 20 PSIa with pure CO_2 to simulate operating conditions. A variety of tubing tubes were used to connect the needle to the pressure transducer and solenoid valve. The leakage rate of each tubing type is shown here. Copper tubing is used exclusively in A-APES because it had the lowest rate of gas leakage (±0.002 PSI/h). PFA, Tygon-PVC, Tygon and Cflex had leakage rates of 0.01, 0.07, 0.08, 0.24 PSI/h, respectively.

Figure S2

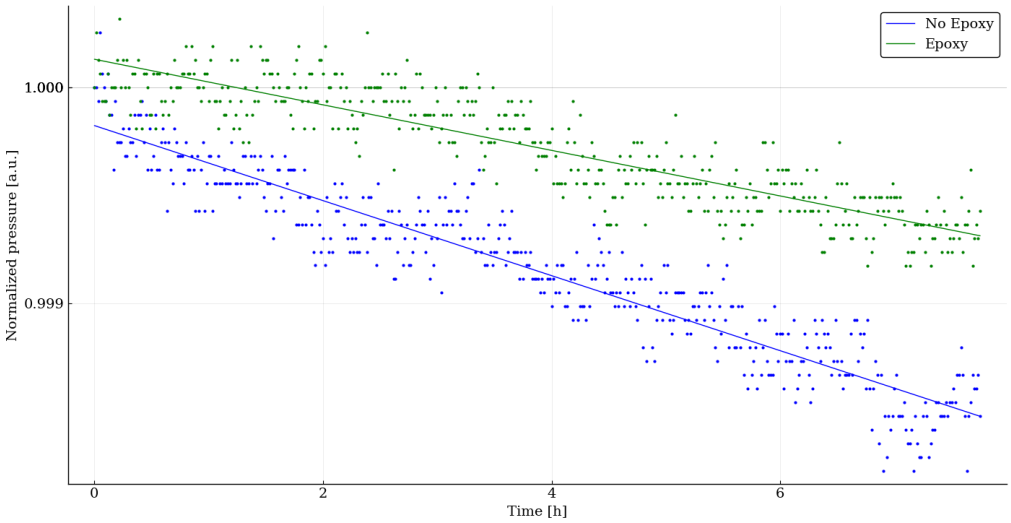

Figure S2: Sealing the connections with epoxy reduced the rate of gas leakage by a factor of 2. The leak rate was tested by measuring the rate of pressure decrease in butyl-rubber stopper sealed 70 mL bottles half filled with 2 mm glass beads and pressurized to 20 PSIa with pure CO_2 to simulate operating conditions. For one test the connections were fastened manually (with a wrench) and for the other test the connections were sealed using epoxy. The rate of gas leakage with and without epoxy was 0.002 and 0.004 PSI/h, respectively, for the assembled system.

Figure S3

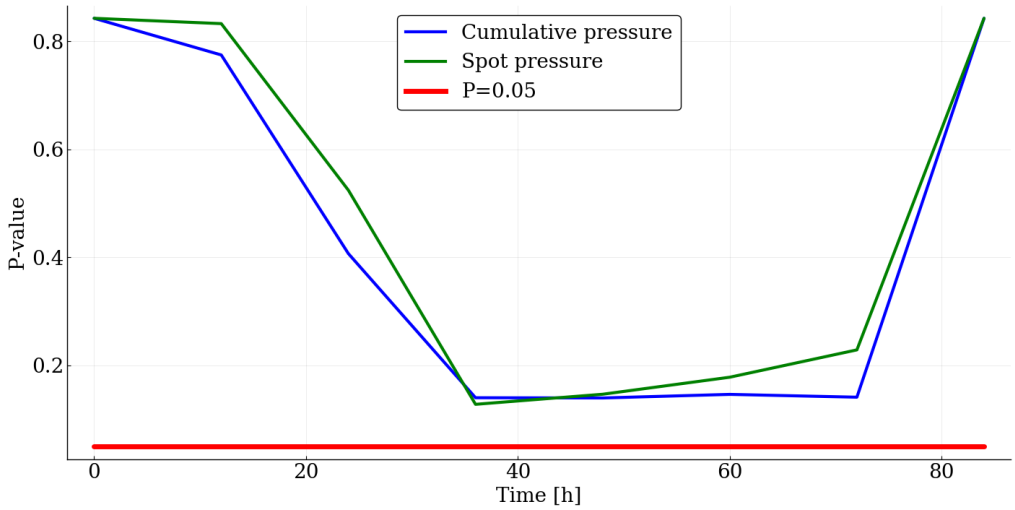

Figure S3: P-value of the differences between the manual and automatic pressure readings over time for both the spot and accumulated pressure measurements were not statistically significantly different. The pressure production measurements of two sets of triplicate *N. lanati* cultures were compared in a side-by-side experiment. Each replicate in both triplicate sets were treated in exactly the same way (2 mL inoculum from the same starter bottle into 40 mL complex media with 0.5 grams of corn stover, see the methods section for more details), except for the measurement method. One set used conventional manual pressure measurements and the other set used A-APES to record the pressure production rate. Both triplicate sets were vented every 12 hours. The differences between both the spot and cumulative pressure profile the p-value, using the unequal variance T-test, was always above 0.05, indicating that there is no significant difference between the pressure measurements.

Figure S4

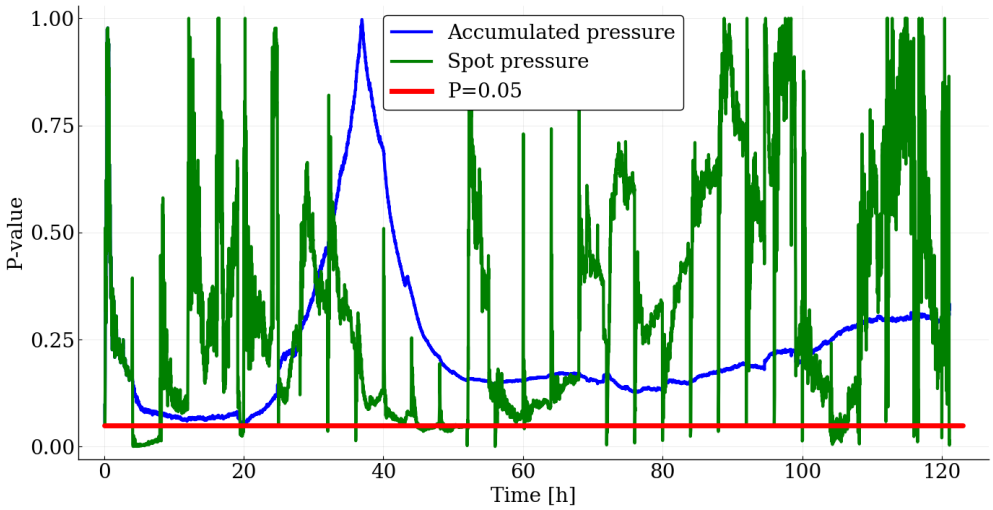

Figure S4: P-value of the differences between two runs of triplicates done at different times illustrate that over 89% and 100% of the spot and accumulated pressure profiles there are no statistically significant differences between two runs done at different times using the same conditions. Two triplicate experiments, using exactly the same experimental conditions (2 mL inoculation of *N. lanati*, 40 mL complex media with 0.5 grams of corn stover, venting every 4 hours and recording pressure measurements every minute, see methods section for more details), were run at different times to gauge the reproducibility of pressure measurements using A-APES. For the differences between both the spot and cumulative pressure profile the p-value, using the unequal variance T-test, was above 0.05 for 100% of the accumulated pressure curve and 89% of the spot pressure curve. This suggests that the readings are consistent with each other.

Figure S5

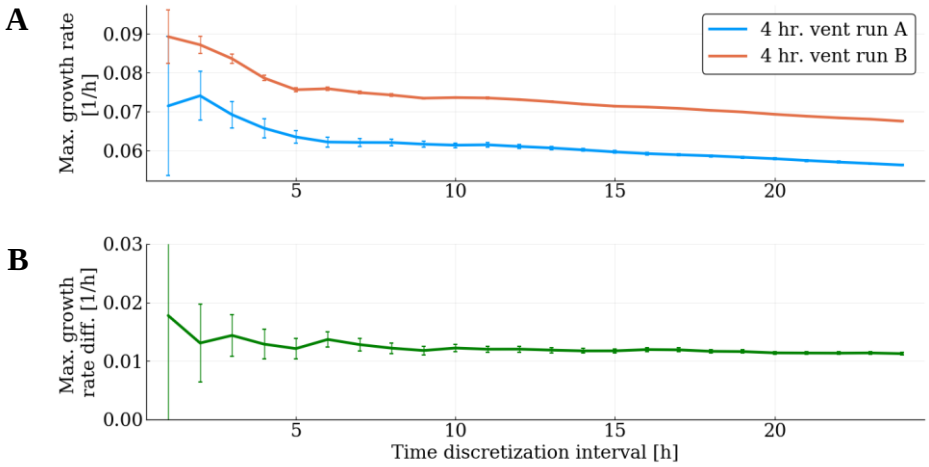

Figure S5: The growth rate calculated from the two different runs of triplicates was significantly different, regardless of the time interval used to calculate them. Two triplicate experiments (run A and run B, respectively), using exactly the same experimental conditions (2 mL inoculation of *N. lanati*, 40 mL complex media with 0.5 grams of corn stover, venting every 4 hours and recording pressure measurements every minute, see methods section for more details), were run at different times to gauge the reproducibility of the growth rates inferred using A-APES. **(A)** The maximum growth rates of each run calculated using different time discretization intervals. **(B)** The differences between these maximum growth rates calculated in (A). The differences are small, with an average difference of 0.01 ± 0.002 1/h, regardless of the time interval used to calculate the maximum growth rate. It is likely that biological noise is the cause of these observed differences owing to the constrained experimental techniques used with anaerobic gut fungi.