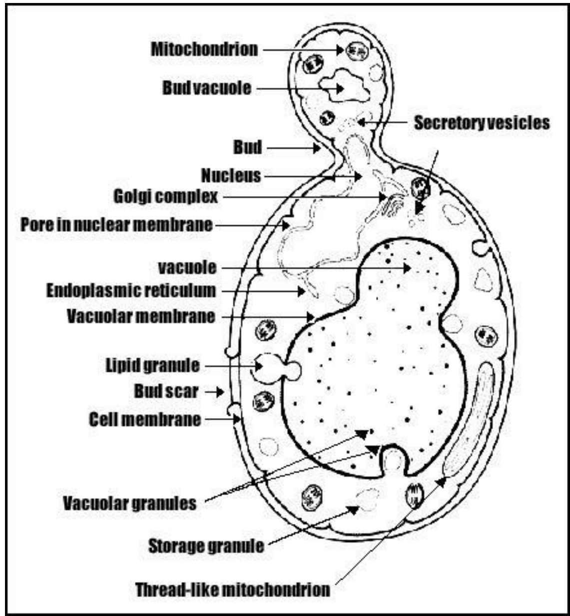
Fermentation NanoLab
Yeast and Bacteria

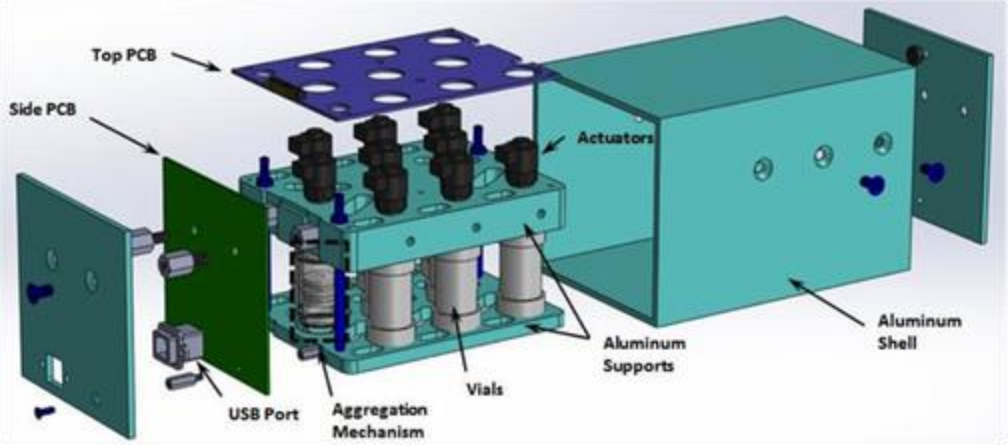
by Glenn Johnson with Logan Sammons

Develop a NanoLab that will allow other researchers to investigate fermentation and related research.

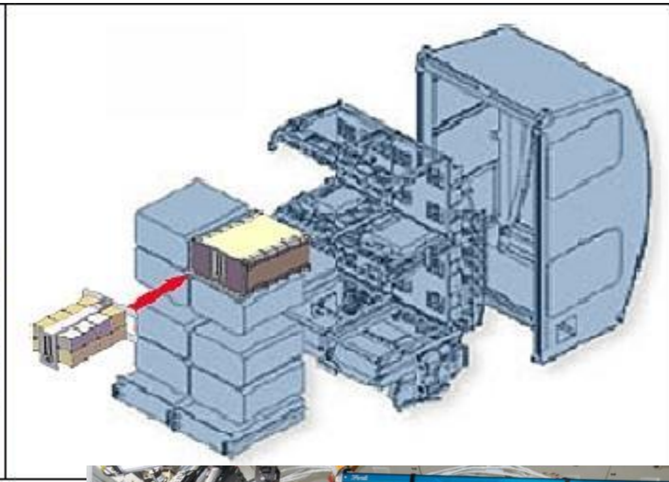


Nanolabs

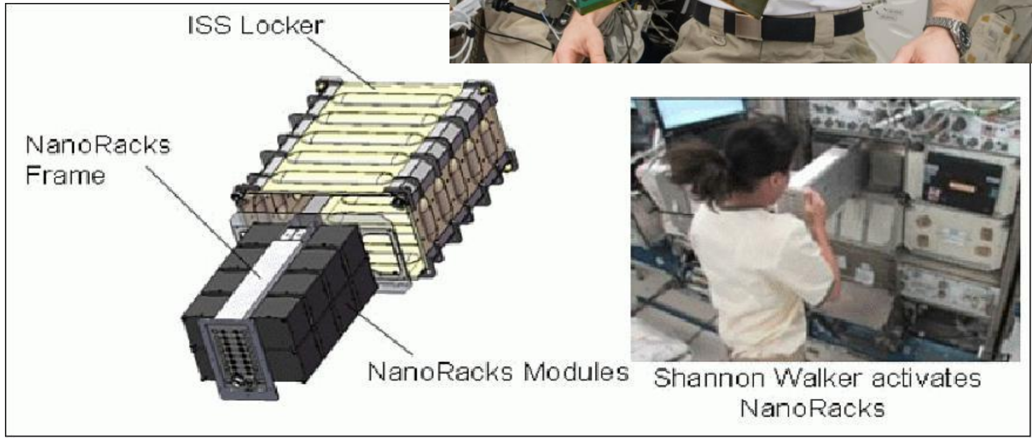
- Nanolabs are small, autonomous, powered experiments that fit inside a specialized ISS Locker that are sent to the International Space Station for testing effects of microgravity on materials, processes, living organisms and many other experiments. These experiments are meant to be relatively cheap so many people and organizations can afford to develop an experiment for the space program. There are specific sizes and power requirements that the experiments have to meet. These are not new and many high schools, colleges and industries have participated in Nanolabs already.
- So far most of these Nanolabs have been specially designed for a very specific experiment. That will probably remain so for many future experiments. However, there are a lot of groups interested in doing an experiment in space but are daunted from doing it because they have to first develop the Nanolab platform for their experiment. This development can take a long time especially if you don't understand all the requirements related to zero-g and the ISS.
- NanoRacks would like to partner with HUNCH students to develop a more generic Nanolab that would allow for a variety of experiments so people could concentrate more on the experiment they want to do rather than on the development of the Nanolab cube for their experiment.



Important General information about all Nano Labs

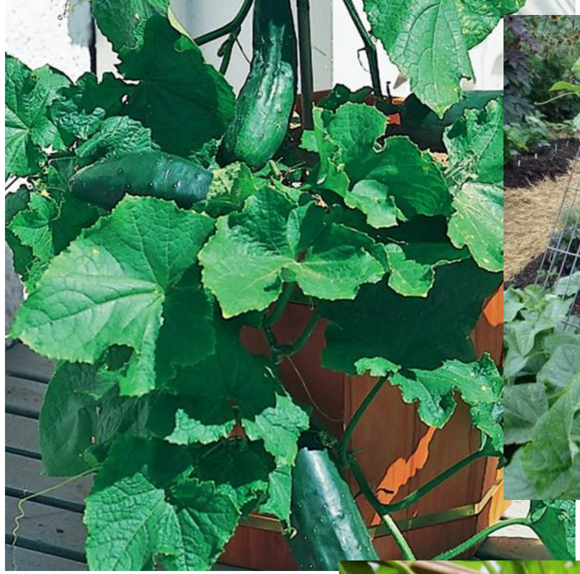


- All Nano Labs (modules) will be installed into a NanoRacks frame —up to 16 Nano Labs to an ISS Locker. This ISS Locker will travel up to the ISS on a supply vehicle and will be removed from the supply vehicle by an astronaut and placed in one of the EXPRESS racks on the ISS where it will be turned on by the astronauts. All of the Nano Labs inside the Experiment box will run autonomously for about 30 days. After that time, the astronauts will remove the Experiment box with all of the Nano Labs inside and place the Experiment box into a vehicle where it will be returned safely to the ground or be burned up in the atmosphere. You should expect that your Nano Lab will never be touched by the astronauts. All of the actions inside the Nano Lab will happen with out human hands. All of the data must come from the Nano Lab and into Space Station computers without human hands.
- All Nano Labs have to be sealed to prevent any contents of one experiment from contaminating another experiment or the living space for the astronauts. This also means there is little if any air exchange between the Nano Lab and the astronauts atmosphere.
- All electronics give off heat when in use. Heat transfer does not happen as fast in zero-g since there is no convection without fans to move the air. Heat transfer by conduction (contact between objects) works well. All of the NanoRacks modules will be cooled by air being blown into the ISS Locker from the EXPRESS rack. The exterior of the Nano Lab is made of aluminum and is a good heat conductor but the more plastic against the interior walls of the Nano Lab the slower the heat will transfer.



NanoLab for fermentation by bacterium and yeasts—

- Although growing a dozen or so plants in space seems to be done fairly easily as NASA and scientists put time and effort into understanding the needs of plants in zero-g, growing enough food in space to feed someone on a regular basis could be problematic at best.
- Consider that if you wanted to feed a crew of astronauts one cucumber per day for a year, it would take around 25 cucumber plants. Not all of them need to be growing at the same time but it takes around 60 days to grow a cucumber from seed to eatable cucumber. Each plant can produce 10 to 15 cucumbers. The crew would have to have at least 4 to 5 plants growing all the time at different stages of development and hope that none die or have problems.
  - What do the astronauts do with the dead plants afterwards getting the cucumbers? On Earth they rot and degrade into the soil.
  - Is pollen or debris from the plant a problem floating around in the space craft?
  - How much volume do these plants require for growth?
  - How much time does the crew need to spend gardening to keep all of these plants cycling so there is enough food for everyone every day?
  - Once the vehicle gets to Mars, what happens to the plants and the grow cycle if they go down to the Mars surface for a couple of weeks? Does someone have to remain on board to tend the plants while others perform the Mars surface mission?
  - This is just for cucumbers. How much volume do I need for any of the other vegetables that need to be grown?
  - How many garden modules do I need to grow enough vegetables for 4 to 6 people?
- As you can see there are quite a few difficulties that will need to be answered for growing food for long trips. There are other things that could be grown in space besides food that could be very helpful to the astronauts. This is not to say that growing enough food in space to feed the crew is impossible. It means that there are many challenges that we have to work through. I suspect that the food that is grown in space is meant to augment the prepackaged and prepared food that is sent.
- The point that is important to take away from this thought experiment is that we need to think deeply about what can be done with the technology that we have and to diversify how we attack the problem.
- That said, not everything that grows and is eatable is a plant. Typically when people think about fermentation they think of beer, wine and other alcoholic beverages. However, while some yeasts are used for making alcohol, bacteria and other yeasts can also be used for making bread, yogurt, cheese, vinegar and even used in genetic research and aid in drug research and development like penicillin.



These are not questions we need to answer now.

Fermentation NanoLab

Problem:
Scientists and Researchers are wanting to send up experiments to the ISS but they don't have enough experience designing labs and understanding the requirements of space hardware. HUNCH wants to make it easier for Researchers to do their science without having to do all of the engineering by making a generic lab that is easier for them to work with. Fermentation is a natural process used in many kinds of food production, beverages as well as in research and pharmaceuticals on Earth including making bread, beer, wine and gene research. Understanding what happens to yeast cells in the radiation levels of the ISS can help researchers understand what happens to human and animal cells in space. There is no doubt that yeast can grow both in liquids and squishy dough in zero-g, the difficulty is how to keep the fermentation from shutting itself down due to the yeast's waste products remaining close to the yeast cells.

Objective:

Design a generic lab for fermenting liquids and solids that can be arranged by different researchers to fit different fermentation experiments.

Requirements:

- Must fit within a 10cm 10cm x 20cm NanoLab.
- Accurately Account for the volume and a variety of locations for the hardware (cabling, camera, sensors, raspberry pi,…) to be installed in the box. Each item may not have a specific location but can be shifted to where the researcher wants them. (think of it like designing a doll house so that a child could arrange the furniture and accessories where they want them to play they want)
- Determine materials for holding the substrate—plastic bag, rigid container, size, shape—cylinder, rectangular
- Determine methods to start the yeast growth after the lab arrives on orbit and turned on.
- The researchers want to be able to arrange and place their growth media in a specific location relative to the camera(s), the water injection, and sensors. It is important that your team makes the NanoLab variable enough that the researchers can locate what they want where they want and be assured that nothing will shift or move during vibrations of launch or while on orbit.
- Needs a simple method for circulating the fluid on a regular or continuous basis so the yeast cells are able to feed without going dormant.

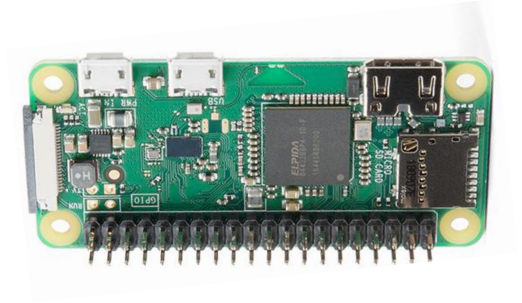
Pointers:

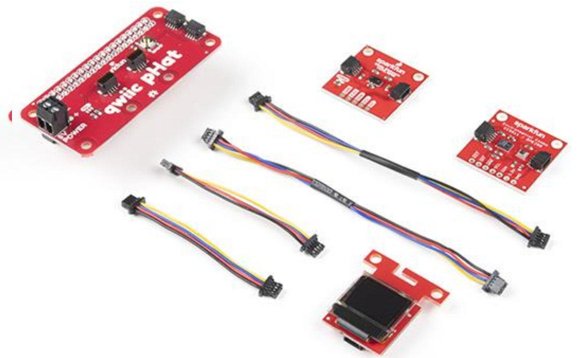
- Is there value to seeing the yeast on a microscopic scale during the experiments or is it better to see the macroscopic picture of the whole mass?
- What kind of lighting is needed for the kind of pictures researchers want?
- Are there colors of light that may be helpful to growing yeast? Infrared, Ultraviolet, visible spectrum, no light?
- Is there a way to shut down the yeast growth at any particular time of the experiments?
- Does food or water needed to be added over the 30 days?
- Know the dimensions of your equipment. Don't forget cabling.
- How do you keep the cables from vibrating out of sockets or getting in the way of the camera?

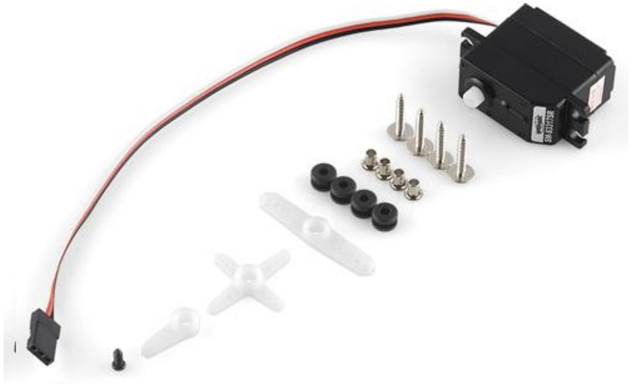
Hardware to consider

- **<u>Fermentation Lab</u>**
- Temperature sensor
- Humidity sensor
- O2 sensor
- CO2 sensor
- Lights
- Camera(s)
- Motor(water distribution)
- Motor (agitation)

These are a few of the sensors and equipment that should be considered for your lab. You are trying to develop a NanoLab that many people could use to do their research with and not have to alter it significantly. It is impossible for your team to be the ‘everything lab’ for every researcher but try to give as many options as possible. There are limits for how many things can be in this confined space and still have enough room for mushrooms to grow. Remember to include cables and motor drivers as needed. There will be cables and they will need to be held down so they don’t vibrate loose during launch and then get in the way of cameras or get cut like the mushroom.



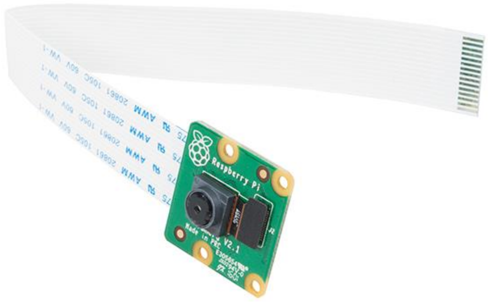


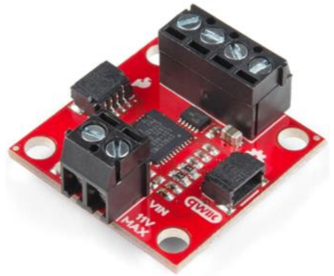


Read through the NanoLab GUI to understand the other team’s responsibilities.

Your job is to show a variety of ways how all the parts can be arranged inside the NanoLab and how they will attach so they don't vibrate out of place during launch. It is important for you to show where the lights, camera and other electronics go and even how it will look but you are not doing the programing. That will be the job of the students who are developing NanoLab Software GUI.
It is your job to work the mechanics of the how the NanoLab will function but still allowing room for the researcher to be creative(kind of like Legos-make it so you can do many things, not just one experiment).

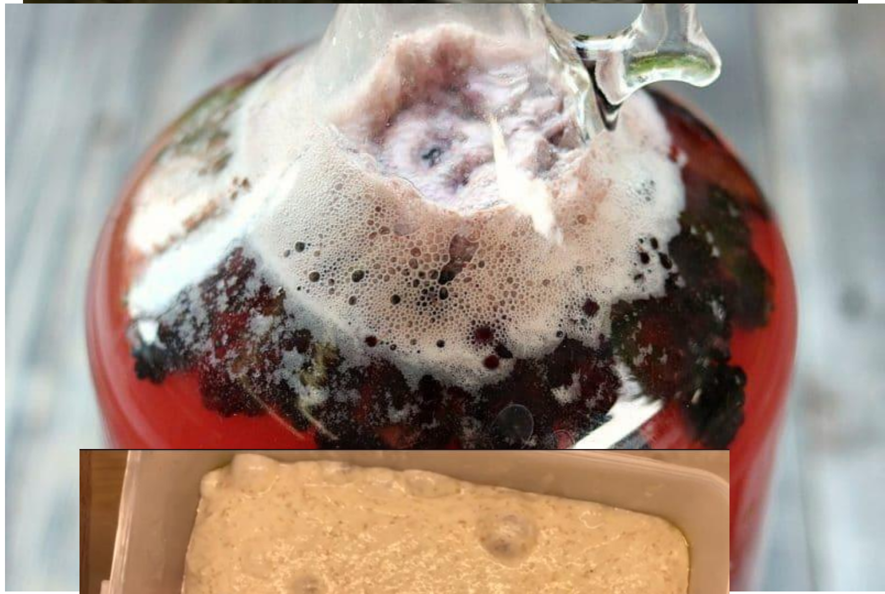
HUNCH can not afford to send everyone a Sparkfun Kit—only the GUI teams. In the table you can see the dimensions of many of the components that researchers may want to use. I found the dimensions on the Sparkfun web pages for the part (usually under features). You can get more detailed information for each of the parts from the Sparkfun website. You may also find other components that you think would be valuable for your NanoLab.





SparkFun Part Number	description	Qty	Notes
PRT-08430	Jumper Wires Premium 6\" F/F Pack of 10	1	155mm +/-5mm long, 26 AWG
TOL-13831	5.1V DC 2.5A Wall Wart (USB Micro-B)	1	AC to DC power supply
DEV-14028	Raspberry Pi Camera Module V2	1	25mm x 23mm x 9mmc, 15cm ribbon cable
PRT-14272	Pi Zero Camera Cable	1	
CAB-14274	MiniHDMI	1	
CAB-14276	USB OTG MicroB Cable	1	
PKG-14011	Large Red Box (9.25x6x2)	1	box it comes in
COM-15052	SparkFun Noobs card Raspberry Pi	1	
DEV-15470	Raspberry Pi Zero WH	1	65mm long x 30mm x20mm
ROB-15451	SparkFun Qwiic Motor Driver	1	25.4mm x 25.4mm x 12.5mm
PRT-16662	Jumper Wires Premium 6in. M/M Pack of 2: Red and Black	1	
WS2812B	SparkFun RGB LED Breakout - WS2812B w/ Headers	1	24 mm x 22mm x 5mm
KIT-16841	SparkFun Qwiic Starter Kit for Raspberry Pi	1	65.00mm x 30.5mm x 10mm
ROB-10189	Servo - Generic Continuous Rotation (Micro Size)	1	42.8mm x 33mm x 16.5mm

Fermenting in Zero-g

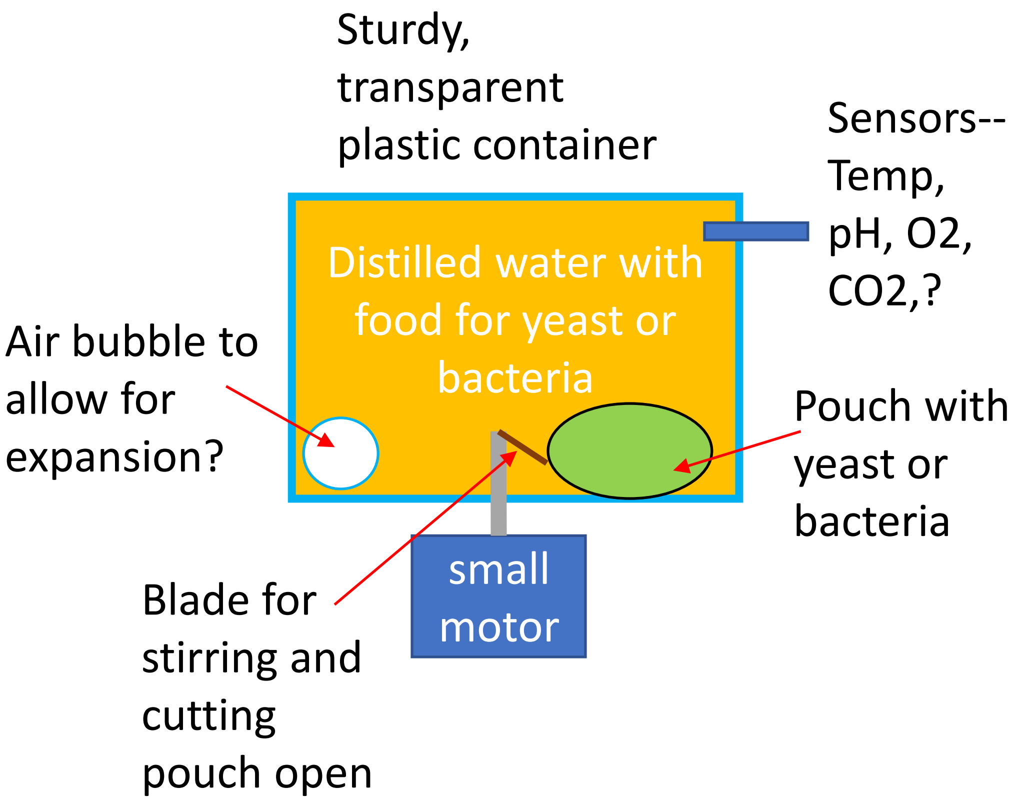


- One of the difficulties with fermentation in zero-g is that the CO2 that is produced doesn’t separate in zero-g like it does on Earth. On Earth, as the yeast digest the carbohydrates and/or sugars in the liquid, carbon dioxide and alcohol are produced. The Carbon dioxide forms bubbles around the yeast cells. As the bubbles get bigger, they bump into other bubbles and coalesce into bigger bubbles and then float to the top of the liquid. They float to the top because the liquid (mostly water) is heavier than the gaseous CO2 so the liquid remains at the bottom of the container and the gas rises to the top of the container. The alcohol forms around the yeast also but as the bubbles move and shift, the alcohol mixes with the liquid.
- In zero-g, there isn’t a top or bottom of the container so when gas is formed, it remains at its location unless there is some other forces that move it. Potentially, the CO2 bubble and alcohol could shut down the reaction since the yeast cells could block themselves away from the nutrients as they produce waste products. There may need to be some method of **stirring and/or agitating** the material so the yeast or bacteria is able to move the waste products away from the cells and still get the nutrients it needs.

Ideas for containing a fermenting liquid

You may come up with a better way to set this up. These are just suggestions.

- a container that can handle an increase in pressure as the yeast generates CO2.
- Is it possible to have a pressure relief valve that only releases CO2 without releasing liquid?
- Start with a bigger container for developing your ideas
- Don't start the experiment until in zero-g
- Could you use a small motor with a blade to cut open a small bag that contains the yeast or the food for the yeast?
- How do you seal the container but still stir the liquid?
- Could a magnetic stirrer be used? Would it affect the electrics that control the sensors or an adjoining experiment? Keep it small?
- What kind of sensors would be valuable
- How do you have the sensors measuring liquid inside without the container leaking as the yeast produces CO2 and increases the internal pressure?
- Camera(s) need to be able to see in
- Is it possible to have more than one container in your box?
- Is it possible to increase the temperature by a few degrees?
- **The key will be experimentation.**



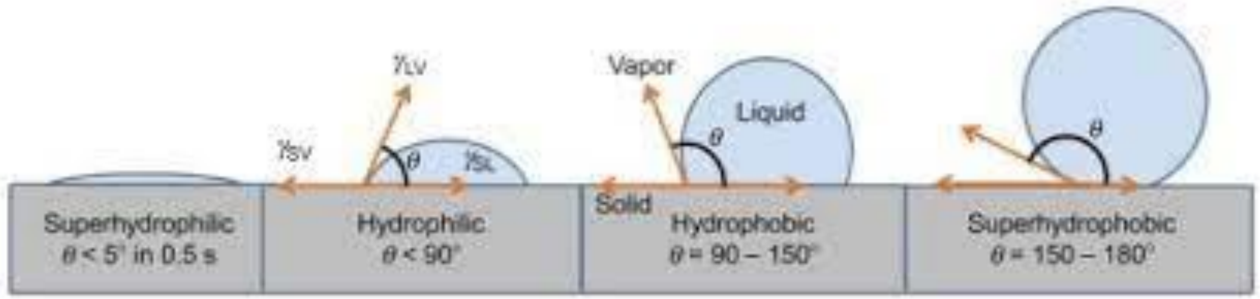
What kind of container could be used for holding the fermenting liquid

Should be transparent for photos and video
Some type of sturdy plastic
Can install sensors in the container
Can hold pressure as the microbes make CO_2

Medical IV bags

Soda bottle blanks

Hydrophilic vs hydrophobic



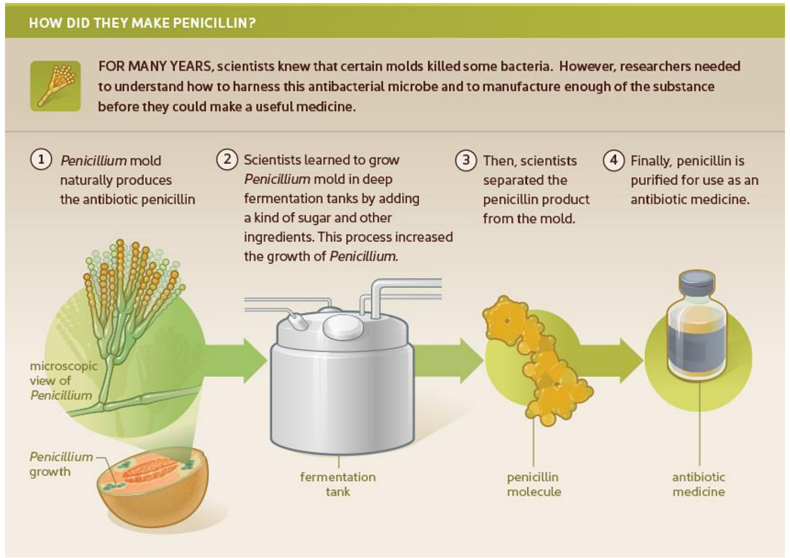
- These properties could be helpful in designing or choosing some of the materials for your NanoLab.
- Hydrophobic materials are things that repel water. Hydrophilic materials are things that attract water. Most materials are somewhere between. A freshly waxed car is more hydrophobic and you can see how the water beads up on the paint. However, a car that has been washed but not waxed, the water sprayed onto it will make a thin coating across the surface—hydrophilic.
- Depending on the solvent and solute, you might be able to find a container material that will contain it by the surface tension.
  - Example—by having a bottle that is hydrophobic you might be able to push water out of the bottle. By having a bottle that is hydrophilic you might be able to have water coat the bottle. By having a bottle that is hydrophobic on the outside but hydrophilic on the inside you might be able to keep it in the bottle

  https://www.youtube.com/watch?v=yxyCLoYfexo

  https://www.youtube.com/watch?v=GcdB5bFwio4

Beading of water shows that the water is being repelled. The unwaxed side (before) is hydrophilic and the waxed side (after) is hydrophobic.

Besides alcohol and bread, what other common products are made using fermentation with yeasts, molds and bacteria?



This is not to suggest that the astronauts will be making any of these products on the way to Mars but only to illustrate that fermentation is an important process used for preserving and making different kinds of food and medicine

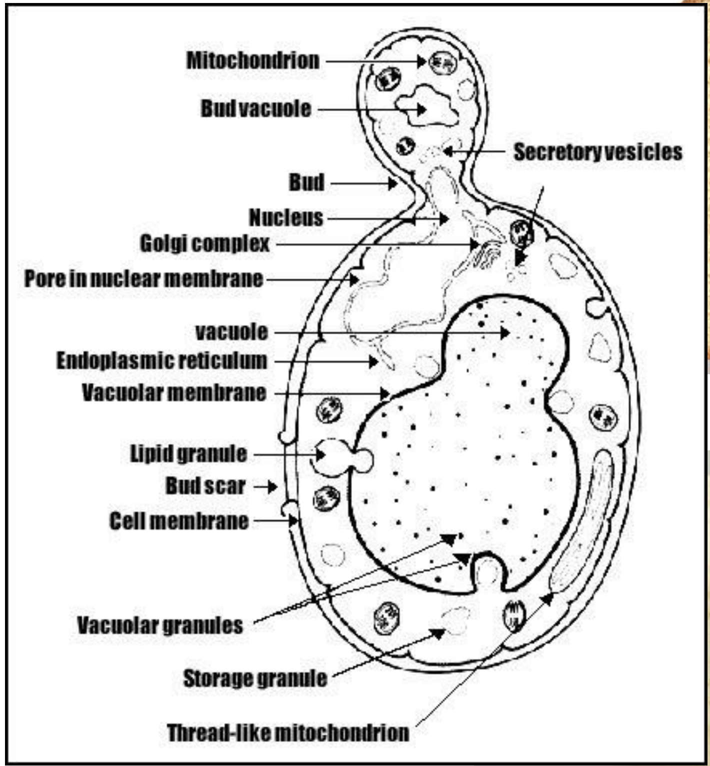
We already know that mold, bacteria and yeasts will grow in zero-g. Right now, astronauts work very hard to prevent mold, fungus and bacteria from growing on the Space Station. Surfaces are often wiped and cleaned to prevent unhealthy strains of microbes from growing and causing health problems for the crew. However, in the future we may find that we need to grow **specific types** of mold, bacteria and fungus to keep the space craft a healthy place to live. So we need to be able to develop methods for studying many forms of life in zero-g. The difficulty is that when bacteria and yeast grown in zero-g, their waste products like CO2 and alcohol can shut down their growth and stop the experiments early. HUNCH is wanting to help the scientists and researchers by making the tools that will allow them to ask and answer questions.

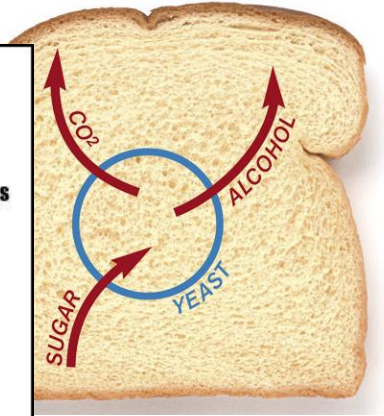
We also know that most plants are symbiotic with different bacteria, yeasts, molds and fungus. Molds, bacteria and fungi are critical in breaking down dead or decaying matter. This is important for plants to pick up those nutrients so that we are able to continually grow plants on our way to other planets. It is possible that some of this research may help with finding the methods of growing more plants in the confined place of a space craft.

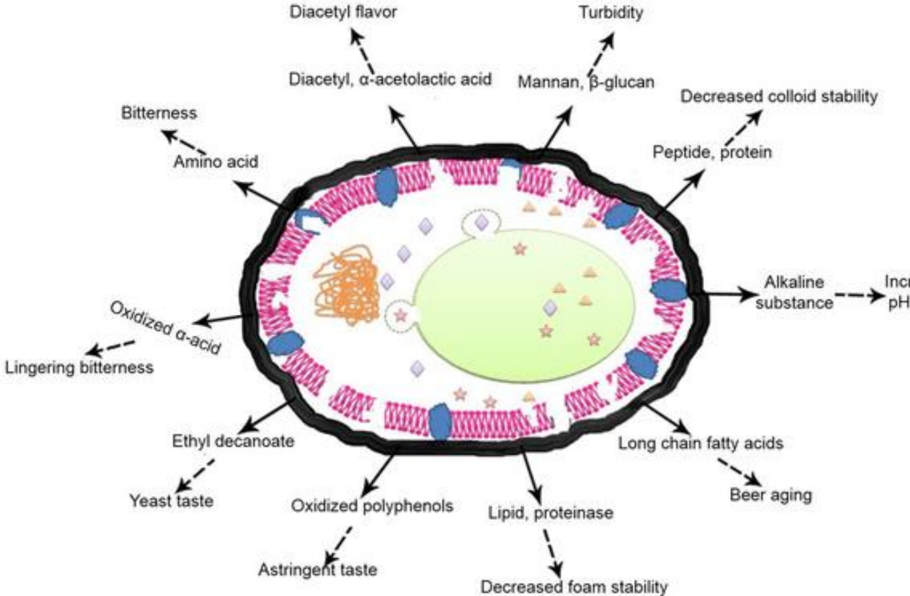
Rising dough?

Would there be reasons for a researcher to want to study something like rising dough? This is a different kind of environment than a bubbling liquid.

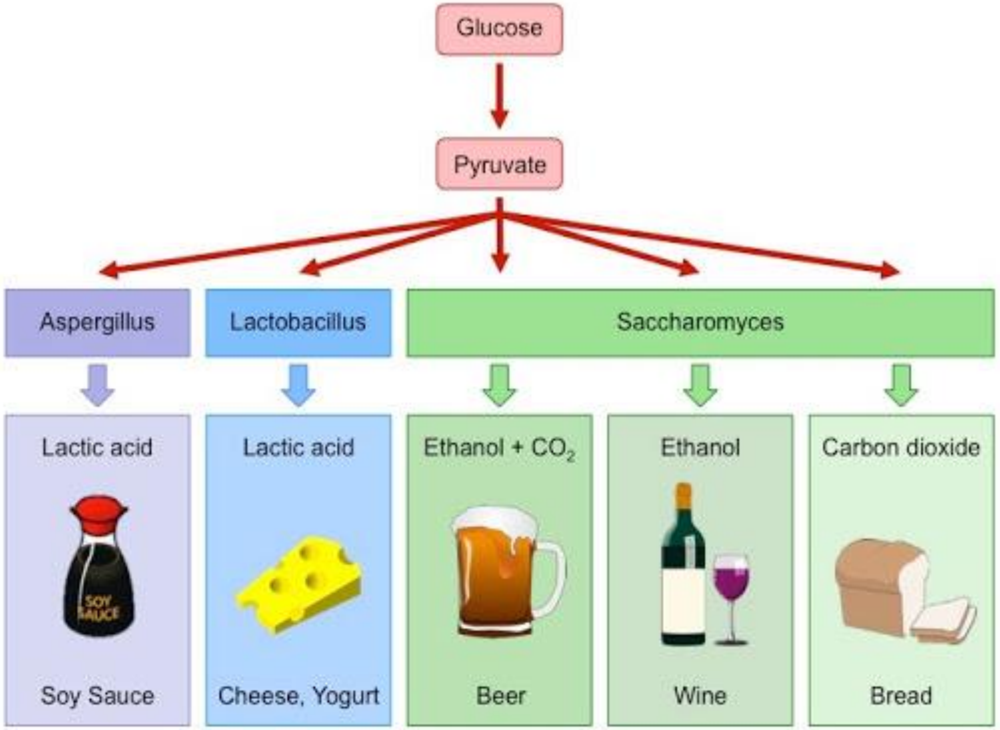
- How long does the yeast/bacteria grow with the limited air supply inside the box?
- How long does a blob of wheat dough grow once exposed to water?
- How much does the blob expand in zero-g?
- Sensors--Temperature, CO2, O2, humidity,
- does the yeast start eating then is choked off from the food and then start again if something jostles the container and shift the bubbles?
- How do you prevent mold from growing on the dough?
- Different types of yeast? Are there some yeasts we want to avoid due to safety reasons?
- What other kinds of media besides wheat dough might be good to grow yeast in?
- How do you hold the blob of dough so it is easy to see it with the camera(s)?
- How many cameras are needed?
- Can I take pictures in different colors of light?
- Is there value to infrared photos?

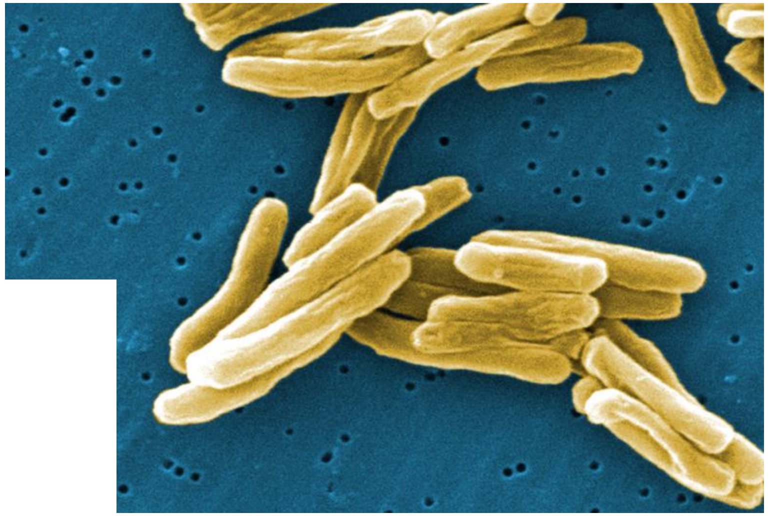


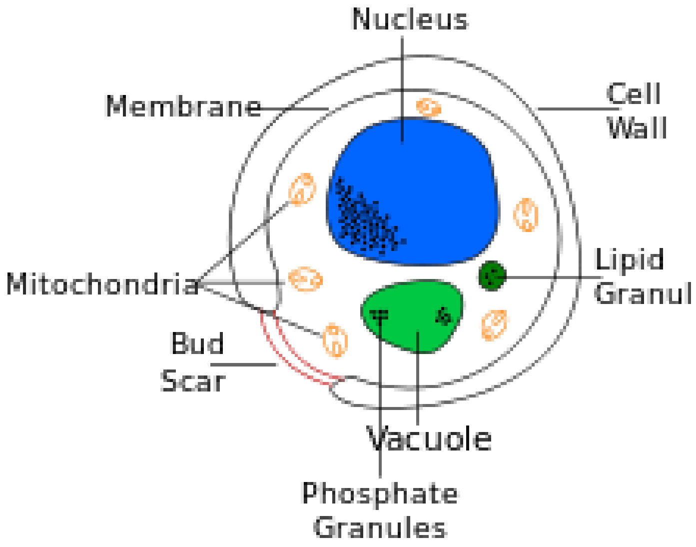





We're not
ALL BAD!!


Glucose
Pyruvate
Aspergillus
Lactobacillus
Saccharomyces
Lactic acid
Lactic acid
Ethanol + CO_2
Ethanol
Carbon dioxide
Soy Sauce
Cheese, Yogurt
Beer
Wine
Bread




Nucleus
Membrane
Cell
Wall
Lipid
Granul
Mitochondria
Bud
Scar
Vacuole
Phosphate
Granules

How might this be valuable for astronauts and space research?

Baking bread on long missions

Alcohol can be used for cleaning, disinfecting, preserving samples.

Vinegar can be used for cleaning, disinfecting, preserving food, samples.

We don't know all of the things researchers will want to study nor do we know how NASA may utilize the yeasts and bacteria as people travel beyond Earth. Some yeasts share genes and genetic material with people and can be used to study human genetics and study drugs. There may be value to studying the effects of space radiation on genes in yeast cells since they can reproduce faster. Researchers may be more interested in the cosmic radiation environment rather than the zero-g environment. Your box will allow scientists and researchers to develop and test their experiments that may lead to new kinds of drugs, methods for preserving food for long space travel, and treatments for diseases both here on Earth but maybe even diseases we find on Mars and beyond. Your objective is to find ways to get the research started. Yes it is possible that some of the research done in your NanoLab may allow astronauts to make cheese, beer and wine on the way to other stars (that's not bad).

- What kind of camera is needed?—visible light? Infrared?
- What kind of lighting is needed for the camera?—colors of LEDs
- How do the gasses change in the container?--sensors
- How does the experiment begin once on the ISS? Initiation of
- How does the experiment end?—
  - will the oxygen in the nanolab get used up?
  - Does the material dry up as the humidity evaporates out of the nanolab?
  - Does the all of the food for the bacteria and yeast get eaten up?
- Is the fermentation effected by light (specific colors)?