A learner-centric microbiology education framework

How to recruit microbes for sourdough baking?

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Storyline

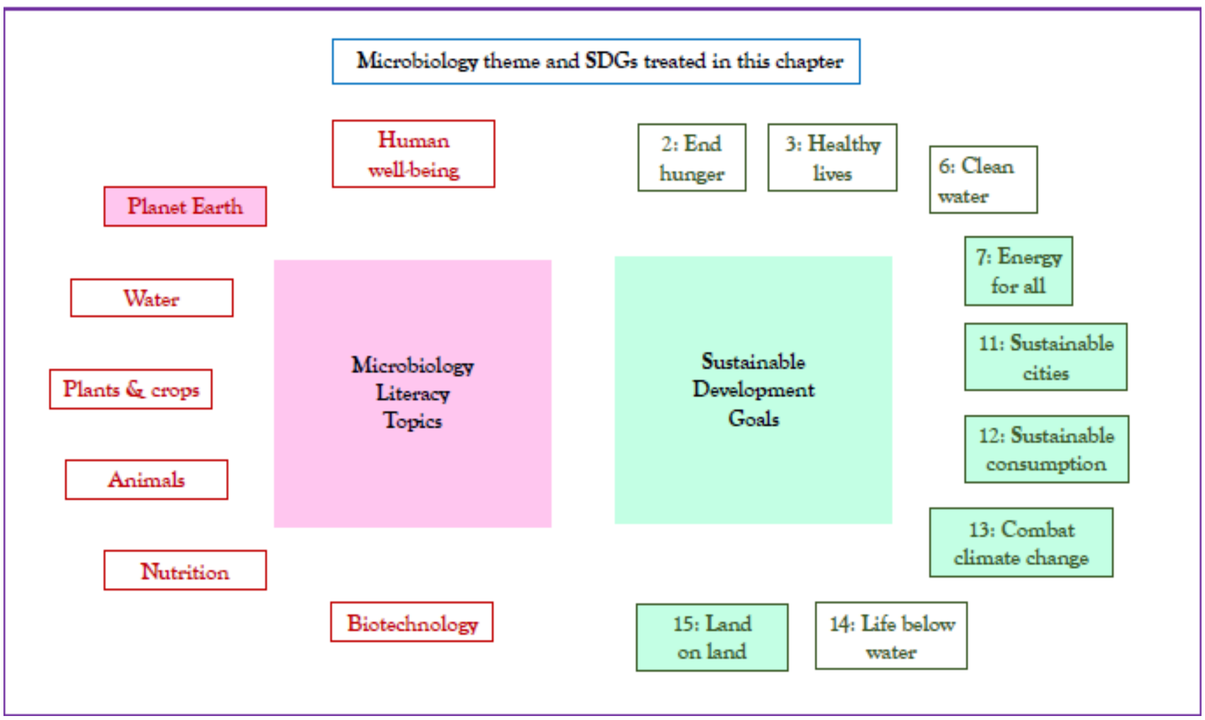
Bread is baked or (steamed) in all temperate climates where wheat or rye are cultivated. Baking bread is a relatively simple process: grains are ground to flour and mixed with water to form a dough. The dough is leavened with yeast before it is baked as a flat bread on a hot stone, in an oven, as, or steamed to steamed buns.

"Leavening" refers to the activity of microbes that produce carbon dioxide in the dough, which is retained in the dough to produce light, fluffy bread. The easiest and fastest way to leaven dough is the use of baker's yeast. Addition of about 2% baker's yeast is sufficient to leaven bread dough in about 90 min. Most of us can buy baker's yeast in the grocery store. Baker's yeast is a concentrated form of a yeast that is called *Saccharomyces cerevisiae*, which prefers to grow in sugar-rich environments including fruit juices and is used to brew beer.

Sourdough is an alternative way to leaven bread. Sourdough is a dough that contains not only yeasts but also lactic acid bacteria, most commonly made with wheat and rye dough. When leavening with sourdough, the bread recipe can be as simple as containing only flour, water and salt – the sourdough is also produced from these three ingredients. The simple ingredient list, however, requires a lot more work than using baker's yeast because the sourdough microbes need to be cultivated in a way that they are active and generate sufficient carbon dioxide. This is not a simple task: When leavening with baker's yeast, about 2% yeast biomass are added to the dough. When leavening with sourdough, the biomass of sourdough microbes accounts for less than 0.1% of the dough weight and the microbes have to be 20 times more active than baker's yeast to produce enough carbon dioxide.

Running a bakery with sourdough as sole leavening agent is thus equivalent of keeping a capricious thoroughbred race horse at peak performance. Below we made a brief reflection on the integration of sourdough and sustainability

The Microbiology and Societal Context



Sustainability: Sourdough bread beautifully reflects the values behind the UN Sustainable Development Goals. Its natural fermentation relies on wild yeast, reducing the need for industrial additives and inputs. By favoring locally grown grains, it supports sustainable agriculture and strengthens rural economies. The slow fermentation process enhances nutrition and digestibility.

Baking sourdough at home or in small-scale bakeries encourages more responsible consumption habits. It also helps minimize food waste, as leftover bread can be easily repurposed into new dishes. Beyond nutrition, sourdough fosters community through shared knowledge and cultural traditions. Its simplicity reduces reliance on highly processed ingredients, promoting cleaner production methods. The mindful, slower process can even lower energy use compared to industrial baking practices. Altogether, sourdough embodies a sustainable, resilient, and community-centered food system.

Before we ask the question on how and why bakers would undertake such an arduous task, let us briefly take a look at the history of baking.

Bread is one of the earliest fermented foods.

The oldest bread we know of was baked 14,400 years ago by a group of hunter-gatherers in present day Jordan (Figure 1). The crumbs of the flat bread that was recovered by archeologists displayed a porous texture, which are clear signs of leavening. The timelines are important: humans knew how to bake bread before they cultivated cereals. This is not too surprising, as cereal grains are neither tasty nor nutritious unless they are processed by grinding, fermentation, and baking or cooking. The excavation of the old bread also reflects that basic baking is not too difficult. Cereal grains are colonized by endophytes - microbes living inside plants. These endophytes include facultative anaerobic bacteria that produce gas when grains, or flour milled from the grains, are kept at ambient temperature for about a day. Why would humans let grains ferment for some time at ambient temperature before consumption? Milling of dry cereal grains is a lot work when it is done manually with simple tools such as grinding stones. The work is lot easier if the grains are steeped in water over night to hydrate the grains and make them softer, followed by wet milling. The steeping, however, also supported growth of microbes and growth, and gas production, continued after wet milling, making production of a somewhat leavened flat bread reasonably straightforward.

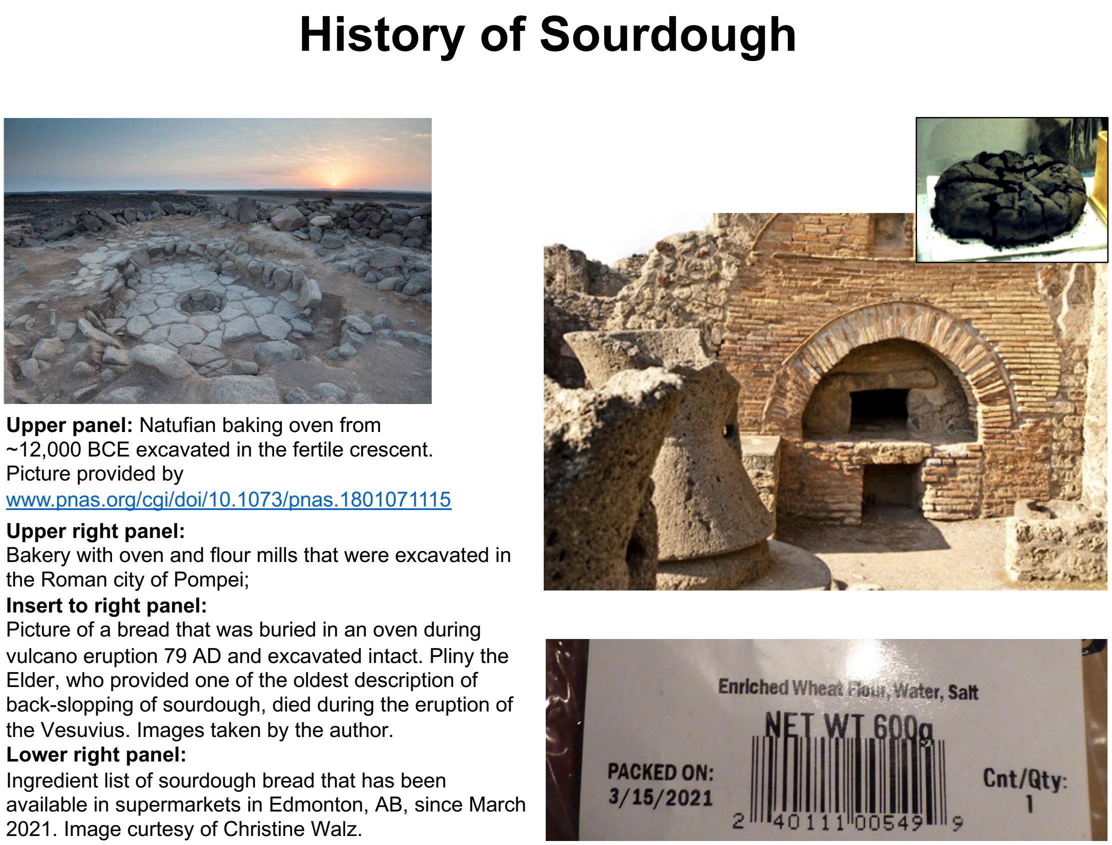


Figure 1. The history of sourdough as detailed in the inlet of the figure

Several (sourdough) bread from later periods of time were excavated in different regions of Europe and the Mediterranean. Egypt, bread was produced at an industrial scale to feed the thousands or workers that were building the pyramids - but we lack detail on how these breads were produced. The next milestone likely dates to the first century. At this time, we know that sourdough for baking was "back-slopped", also referred to as "refreshment" or "feeding" by sourdough bakers, i.e. a small piece of ripe sourdough was used to start the next sourdough (Figure 1). As we will see later, this fundamentally changes which microbes populate the sourdough and how useful they are for baking. How do we know? One of the best-known Roman technical writers, Pliny the Elder, writes around

70 CE in his Natural History that "it will usually suffice to take a small part of the dough from the previous dough as it is commonly known that it is an acid compound that is responsible for fermentation". Reference to sourdough in the New Testament, which was written at approximately at the same time in the same jurisdiction, also documents that the writers expected that their audience is familiar with the use of back-slopped sourdough as leavening agent. Paul Feinstein, who wrote a documentary for the BBC on the world's oldest sourdough in 2022 (https://www.bbc.com/travel/article/20220711-the-worlds-oldest-sourdough), indicated that it is possible or even plausible that some sourdoughs have been maintained for 2000 years but the oldest documented age of contemporary sourdoughs is about 150 years.

The next milestone in the history of baking was Louis Pasteur's discovery of yeast as fermentation organism in wine, beer and bread in 1857 and the commercial production of baker's yeast in Vienna about 15 years later. After the Second War, baker's yeast was grown with high purity on molasses as substrate – the use of sourdough as leavening agent was no longer necessary and most wheat bread was produced with baker's yeast as only fermentation organism. Only in countries where rye bread accounted for a substantial portion of the bread – most countries in Central, Northers and Eastern Europe – continued to use sourdough to achieve acidification of rye doughs, which is necessary to achieve an acceptable quality of rye breads.

Fast forward to the last change of why and how sourdough was used in baking applications: since about 2010, sourdough fermentation has been increasingly applied in wheat baking with the aim to improve flavor, texture, and the mold-free shelf life of bread. A brief overview on the benefits of sourdough in general, or specific sourdough microbes is provided in Table 1. In addition, the use of sourdough allows to produce delicious bread while listing flour, water, salt as sole ingredients (Figure 1) – such a short list is preferred by many consumers. The Covid-19 pandemic enhanced the use of sourdough for baking at the household level – one reason was the shortage of baker's yeast in many supermarkets after the lockdowns in the first half of 2020.

Table 1. Contribution of sourdough microbes to bread quality. Entries with grey shading require metabolically active lactobacilli during proofing. The table was modified from Gänzle et al.,

Metabolite / metabolic pathway	Impact on quality	Organisms
	Carbohydrate metabolism	
Formation of lactic acid	Increased acidity; sour taste and inhibition or activation of cereal enzymes	All lactic acid bacteria
Formation of acetic acid	Odor and inhibition of mold growth	Heterofermentative lactic acid bacteria
Formation of CO_2	Leavening	Heterofermentative lactic acid bacteria and yeasts
Formation of extracellular polysaccharides	Improved bread volume, delayed staling	Strain- or species specific in lactic acid bacteria
	Protein and amino acid metabolism	
Release of amino acids from peptides and proteins	Taste compounds and precursor compounds for odor volatiles	Cereal enzymes (proteolysis), lactic acid bacteria (conversion of peptides to amino acids)
Conversion of glutamine to glutamate	Umami (savory) taste	All lactic acid bacteria
Conversion of arginine to ornithine	Precursor to formation of rusty smelling compounds during baking	Species-specific in lactic acid bacteria
Conversion of glutamate to γ-butyric acid	Bioactive, relaxing compound	Strain- or species specific in lactic acid bacteria

Reduction of disulfide- to sulfhydryl groups	Impact on dough elasticity, bread volume, removal of allergens and potentially improved protein digestibility	Species-specific in heterofermentative lactic acid bacteria
Formation of γ-glutamyl peptides	Kokumi activity: improved mouthfeel and long-lasting taste	Species-specific in heterofermentative lactic acid bacteria
Formation of odor volatiles from amino acids	Improved flavor	Strain specific in yeasts
	Lipid metabolism	
Reduction of odor volatiles from lipid oxidation	Improved flavor	Heterofermentative lactic acid bacteria
Conversion of unsaturated fatty acids to antifungal compounds	Delayed mold growth on bread	Strain- or species specific in lactic acid bacteria
	Others	
Conversion of phenolic compounds	Flavor, bioactive compounds	Strain- or species specific in lactic acid bacteria and yeasts

How to we recruit microbes for sourdough baking?

The microbes in sourdough are recruited from the raw materials, the environments, and in specific cases from commensal microorganisms that have adapted to animals. The principles of community assembly are depicted in Figure 2. If a sourdough is started with flour and water, most of the microbes in sourdough originate from the grains. The presence of other microbes is limited by transport of organisms over space, which is referred to as “dispersal” in microbial ecology. The most abundant microbes grow first, the growing acidity will select for the most acid resistant microbes, which prevail after 1 – 2 days of fermentation. The two lactic acid bacteria *Levilactobacillus brevis* and *Lactiplantibacillus plantarum* were isolated most often from such spontaneous sourdoughs. If such a spontaneous sourdough is propagated by continuous back-slopping, *Saccharomyces cerevisiae* will join the lactic acid bacteria and the sourdough can be used as leavening agent to bake bread after about 10 back-slopping steps.

When back-slopping continues over long periods of time – as outlined above, some sourdoughs have a history of well over a hundred years of continued back-slopping – the dispersal limitation is eliminated: after dozens or hundreds of back-slopping steps, contamination from even unlikely sources such as wild animals, flowers or other environmental or plant sources becomes a certainty. If a sourdough is propagated for a sufficient length of time, the sourdough yeasts adapt to their new environment. Just like domesticated horses and dogs differ from their wild ancestors because humans selected for specific traits over many generations of breeding, yeasts become domesticated in response to the selection. In ecology, this process is referred to as "speciation" (Figure 2).

Which microbes do we find in back-slopped sourdoughs? It depends on how bakers maintain their sourdoughs. Above, I compared the task of maintaining a sourdough at peak performance in a bakery that bakes daily to the training of a capricious thoroughbred race horse. The best solution requires fermentation schemes that are so similar globally that only two organisms, *Fructilactobacillus sanfranciscensis* in association with the yeast *Kazachstania humilis,* are in most of these sourdoughs. Despite the reference to the city of San Francisco, U.S., and the Central Asian country Kazakhstan, these organisms are the same all over the globe: in China, where sourdough is used for production of steamed buns, in the Middle East and the Mediterranean, where sourdough is used for production of flat bread, and in all European countries including their emigrant offspring in the Americas and

Oceania. Chinese steamed buns, Italian Panettone, Finish rye bread and the San Francisco sourdough bread taste and smell very different but they are produced with the same microbes.

Sourdoughs that are fermented in Nordic countries for acidification of rye doughs are fermented to obtain a high level of acidity rather than leavening power. This is best achieved by using a high (5 - 45 °C) temperature and long (1 – 4 days) fermentation times. Most of these sourdoughs are populated by organisms of the genera *Limosilactobacillus* and *Lactobacillus* that are associated with the intestine of humans and animals. For some sourdough isolates, it is quite certain that they originate from the intestine of a rat or a mouse: the strains are genetically identical to mouse isolates and colonize mice just as well.

This sounds disgusting or even risky for human health as the intestine also contains pathogenic bacteria? Let us take a closer look at how maintain a sourdough for a month or so turns something disgusting into something delicious. On their way from the field to the mill and to the flour used bakeries or sold in supermarkets, grains or flour are almost certainly contaminated by animals and their microbes. The Canadian food regulations specify a maximum number about 100 milled insect fragments and up to 3 rodent hairs per 225 g of wheat flour, reflecting that it is close to impossible to produce flour without a low level of contamination from rodents or insects. What happens if wheat flour that is contaminated with enough animal microbes is used to back-slop (or feed) a sourdough? The amount of mouse or rat intestinal material or other that is transferred is likely very small –fragments of insects or rodent hairs represent approximately a millionth of a gram but this amount is sufficient to transfer about thousands or microbial cells including dozens of cells of very useful lactobacilli. After the first refreshment, the lactobacilli grow but the other, undesirable microbes do not grow or even die: after a few back-slopping steps with 10% old sourdough, the initial thousands of undesirable cells all diluted out and completely gone. If the lactobacilli from mouse or rats are more competitive than those microbes that were already present in the sourdough, they will become dominant after 10 – 20 back-slopping steps.

The increased use of sourdough in industrial baking, and by amateur bakers at the household level also increased number of yeasts and lactobacilli that were detected in sourdough. As outlined above, *why* sourdough is fermented dictates *how* it is fermented and different aims and fermentation conditions thus select for different fermentation microbes. Each bakery and each home baker have their own way of using (and storing) the sourdough. This diversity of fermentation and storage parameters also results in diverse fermentation microbes. Currently, more than 100 of the about 340 species of food-fermenting lactobacilli (*Lactobacillaceae*) well as dozens of different species of yeasts were isolated from more than one sourdough. We continue to learn: when we sample from bakeries that ferment with radically different conditions, we are likely to find very different lactobacilli and yeasts.

It is difficult to bake sourdough at home or in a school experiment?

Depending on the learning outcome and the experimental design, experiments related to the use of sourdough in baking cannot only teach a very basic food preparation technique but also illustrate basic principles of ecology. A brief guide of the most important principles for sourdough maintenance and sourdough baking is provided as "sourdough paramedic" in Figure 2.

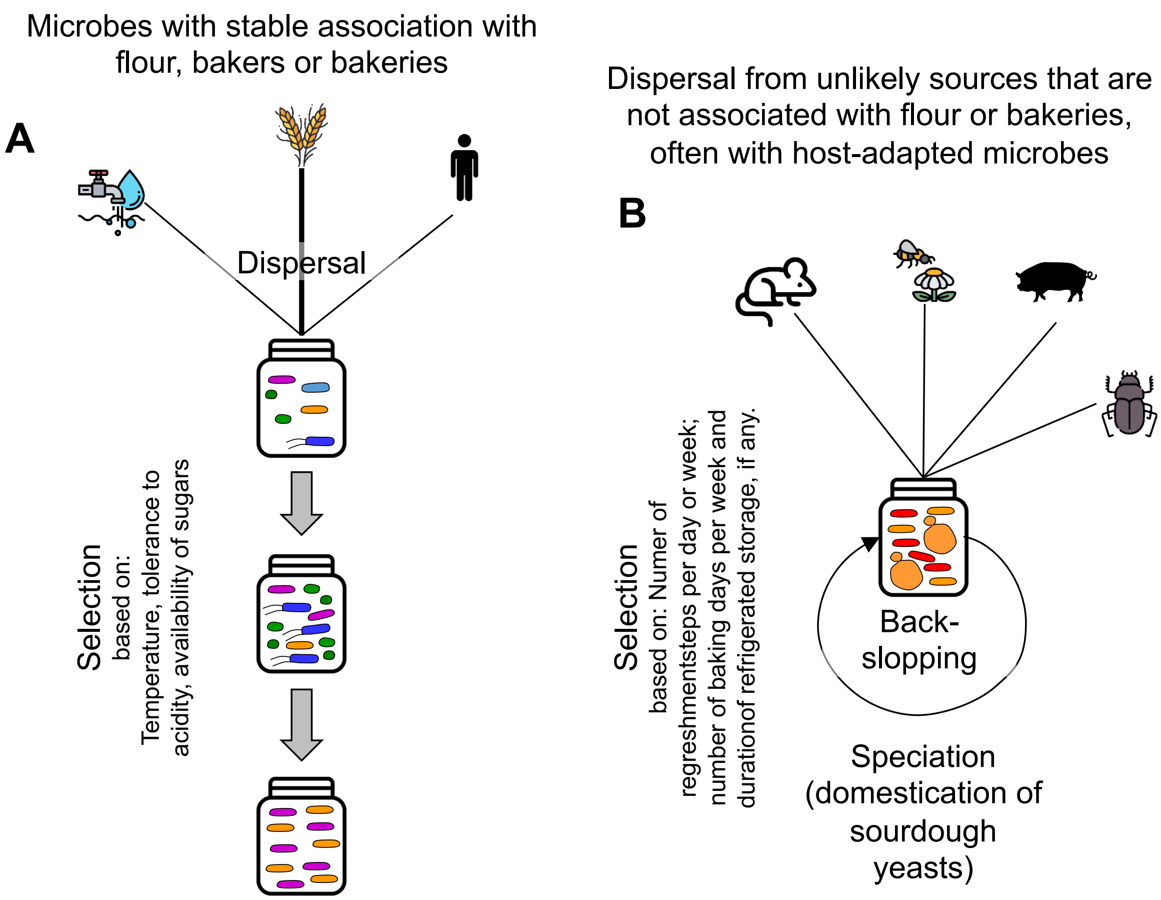


Figure 2. Community assembly in sourdough fermentations. Panel A. Microbial communities in spontaneous sourdough fermentations that are started with flour and water only originate from the flour, the bakers or bakeries are determined by dispersal and selection. Most spontaneously fermented foods undergo a characteristic succession of fermentation microbes that reliably is dominated by beneficial lactobacilli at the end of the fermentation. Panel B. Community assembly in back-slopped sourdoughs. Here, community assembly is not limited by dispersal and selection is the major driver for the assembly of microbial communities. The microbes that dominate in back-slopped sourdoughs are fairly uniform in sourdoughs that are maintained as leavening agent in artisanal bakeries but show significant variations when maintained in households by amateur bakers. Microbes in back-slopped sourdoughs are often adapted to insect or vertebrate hosts. Some sourdough yeasts are known to be domesticated after decades or centuries of continuous propagation. Modified from Gänzle et al., in preparation, with icons from https://www.flaticon.com.

Safety first: The most significant risks are wheat allergies, which are more frequent in children than in adults, and gluten, which causes severe intestinal inflammation in individuals with celiac disease. These risks can be addressed by using flour from sorghum, millet or pseudo-cereals such as quinoa for any experiments; the breads will be very different from wheat bread but the principles of recruiting microbes for baking can be observed.

If commercial flours are used and a basic level of hygiene and good food handling practice is adhered to, it is extremely unlikely that a sourdough that is started with flour and water results in microbial foodborne disease. In addition, bread is baked before it is eaten so all sourdough microbes are killed.

If animal material is handled to test the role of animals to transmit fermentation microbes, risks associated with zoonotic transmission of pathogens are have to be addressed by working with appropriate laboratory infrastructure to safely work with potentially pathogenic microorganisms, which may or may not be available in many high schools, and with an instructor who has experience working in a biosafety level 2 laboratory.

Will the experiment result in the perfect loaf of sourdough bread? Likely no. When a spontaneous sourdough is started, the first fermentation batch may smell a bit funny because lactobacilli have not quite taken over yet – keep on feeding for a few more refreshment cycles. A good sourdough also looks and smells a bit funny after being stored for too long in the fridge – again, this can be rectified by a couple of refreshment cycles. In addition, sourdoughs may have "bad hair days", particularly when used by inexperienced bakers, and show too little activity to leaven bread. If you are not sure that your sourdough is bubbly enough, add a little baker's yeast – 1 g yeast addition for 100 g flour will improve leavening and keep the proofing time short even for a sluggish sourdough. If a sourdough dies after neglect in the fridge or the kitchen counter, start it again – it will take a week to get a new one active again and a bit of addition of baker's yeast for baking.

Can the roles of dispersal, selection and speciation be tested in a simple experiment that is performed at a school? These hypotheses can be tested in a basic way.

To determine the role of dispersal, different sourdoughs can be started with only flour or water, or after addition of a small amount of different (food-grade) plant materials (chopped vegetables or fermented pickles, different flowers or berries, fermented food with live fermentation microbes). The sourdoughs will likely look and smell very differently after the first 1 – 10 fermentation cycles, have a different volume after a day of fermentation and will differ in their suitability for use as leavening agent. The level of acidity can additionally be quantified by pH indicator strips or by titration.

The role of selection can be tested by using the same starter for back-slopping with using different fermentation conditions, e.g. by feeding two a day versus every 2 days, or by fermentation at 20, 30 and close to 40 °C. Again, the simplest parameters that can be assessed are look (volume) and smell, the level of acidity and the suitability for use in baking.

The role of speciation or domestication is much more difficult to test – we don't know the timelines for speciation and even an entire school year is unlikely to suffice. In addition, advanced microbiological methods including genome sequencing are required to detect reliable indicators.

To deep in the science behind the sourdough from microbiology to nutritional value

https://www.sourdoughinstitute.com/research-papers

https://thebaker.science/en/

https://www.sourdough.co.uk

https://www.theperfectloaf.com/

https://thesourdoughjourney.com/

https://www.theclevercarrot.com/

https://www.amazon.com/Artisan-Sourdough-Made-Simple-Handcrafted/dp/1624144292?&_encoding=UTF8&tag=emilieraffa-20&linkCode=ur2&linkId=048b2b65b988998abbf1c5084bdd6f45&camp=1789&creative=9325

https://www.kingarthurbaking.com/recipes/sourdough?menu%5Bcategory_lvl0%5D%5B0%5D=Sourdough