Steam sterilisation in spawn production

Mushroom spawn must be axenic. That means that it only contains one species and is not contaminated with any foreign organisms. Any mistake at this point creates disasters further down the line. There is only one proper way to produce axenic spawn: steam sterilisation of the substrate followed by aseptic inoculation.

By Kasper Moreaux, Mycelia

Steam sterilisation has overwhelming advantages and is universally used in spawn production. This article briefly explains the production of aseptic spawn recipes, whether in liquid, solid or semi-solid state. Aseptic means that after the treatment, it contains zero foreign organisms.

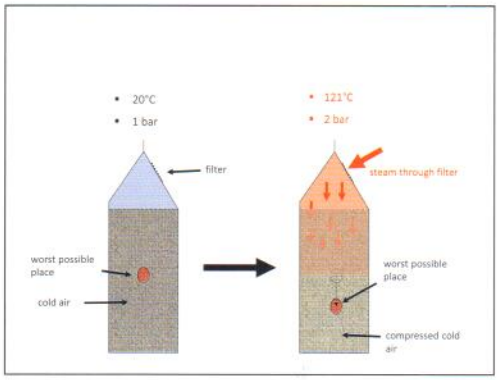

Fig 1. Compression of gases in bag during autoclaving.

Steam sterilisation of a substrate is a very complicated science and is usually underestimated. The proper heat treatment of a substrate starts with choosing a production method: bulk, in bags, liquids, which all require different steam sterilisation cycles. But they have a common goal: every particle of the substrate must be sterile. To achieve this, each particle must reach a temperature plateau of 121°C or more for a certain time. The amount of time at this plateau depends on the temperature and on the initial spore load of the substrate, which is expressed in a so-called "Fo-value". In most spawn substrates, 15 minutes is enough. In bulk and liquids, this is reasonably straightforward, as gases are easily evacuated. In spawn bags, bottles and other containers however it is much more complicated because gases get trapped inside the bags (see fig. 1).

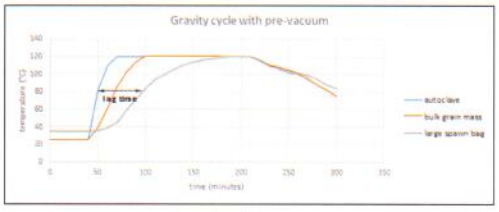

Fig 2. Gravity cycle.

It is important to keep the sterilisation time at 121°C as short as possible. At these temperatures, several chemical reactions take place, such as caramelisation and mayar reactions. These reactions are detrimental for the quality of the spawn: originally available food elements are transformed into indigestible complexes and toxins. The spawn producer should apply the shortest possible sterilisation time, while ensuring that all particles are sterilised. The latter can only be determined with the aid of probes.

There are three main sterilisation cycles in the production of spawn: the gravity cycle, the liquids cycle and the vacuum cycle/pulsating cycle.

The gravity cycle

The simplest version of a sterilisation cycle is a gravity cycle: the pressure and temperature in the autoclave are increased until they reach the sterilisation plateau at 121-123°C as displayed in fig. 2. This plateau is held stable until all particles are sterile. Then, the temperature and pressure are decreased.

The basic idea behind this cycle is that during the first phase, the air and other gases are replaced by steam by means of gravity and pressure. They must be removed due to the negative effect they have on the sterilisation temperature (see fig. 3) During replacement, the gasses are exhausted through the opened venting valve. This is important, because air and other gases act as insulators, so the temperature of the steam cannot be transferred to the particles. When steam has replaced all gases, the venting valve is closed, allowing the pressure to build up to the plateau. During the plateau, a so-called bleeding valve at the top of the vessel allows for continuous evacuation of gases which

form during autoclaving. At the end of the autoclaving cycle, cooling is achieved by slowly releasing the steam through the venting valve, by allowing the steam to naturally condense inside the vessel or by sucking it out mechanically. Just before the pressure has reached atmospheric pressure, the venting valve is closed. The volume of the steam, as it condensates and changes into water, will shrink to 1700 times less than its gaseous size. This creates an immense vacuum inside the vessel, which must be replaced with sterile air. So, at the same time when the venting valve is closed, the sterile air inlet valve must be opened. Important: if at this point, non-sterile air is allowed into the autoclave, contamination of microorganisms may spoil the entire autoclave.

When sterilising a grain substrate in a bulk vessel, the simplest version of the gravity cycle can be utilised, as explained above. The plateau at 121°C must be kept for 40 minutes or less, depending on the substrate recipe. Optional extras that shorten the cycle are: 1) creating a pre-vacuum before pressure increase to eradicate all gases before steam is injected, and 2) using a post-vacuum after cooldown to speed up the cooling process.

When sterilising grain or substrate spawn in bags - or bottles, boxes or other recipients - the gravity cycle is not the best option. When steam is injected into the autoclave, it is supposed to gradually replace the air in every part of the substrate during the pressure build-up. In an open mix such as a bulk vessel, this is easy as all pore spaces are open and there are no obstructions anywhere. But in an autoclave filled with bags the situation is totally different.

The bags are being compressed as the steam builds up the pressure, but there is no free exchange of steam and air between the bag and the surrounding space in the autoclave. The gases inside the bags get trapped and act as an insulator: where they prevent the steam from penetrating the substrate, the heat transfer is much less efficient. The heat will only slowly pass through the plastic to reach the substrate particles on the inside. In large bags filled with grain, this process can take as much as 240 minutes. As a result, the temperature inside each bag changes gradually, with the outer layers heating up faster. This temperature gradient causes quality differences inside each bag and an overall quality drop. There are only two possibilities to get rid of this problem: 1) use smaller bags or 2) apply a vacuum sterilisation cycle.

The liquids cycle

The liquids cycle is used to sterilise liquids. Examples are liquid spawn substrates and agar bottles. It is comparable to the gravity cycle, with one difference: during heating and cooling, the temperature must be gradually and slowly increased and decreased. If not, the bottles will burst and/ or the liquid will start to boil violently, causing serious problems.

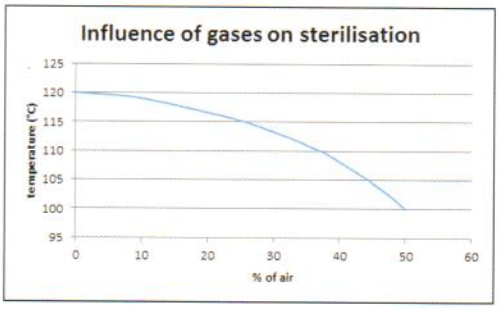

Fig 3. Influence of air on sterilisation.

The vacuum cycle

The vacuum cycle or pulsating cycle (see fig. 4) is a rather complicated sterilisation cycle. It has been developed specifically for sterilising porous and bagged goods, such as pre-bagged spawn substrate. The cycle works as follows: the pressure is increased and decreased in a number of pressure pulses, followed by a stable maximum temperature plateau at 121°C or more. During the pulses, the air and gasses that are present inside the bags - or bottles, boxes, etc - are replaced by steam by force. This way, the time for every particle to reach 121°C is shorter and the temperature plateau can be much shorter than during a gravity cycle: 30 minutes or less, depending on the type of substrate and packaging, and positioning of the bags in the autoclave.

Fig 4. Vacuum sterilisation cycle.

Without trapped gasses, there is no temperature gradient inside the bags. This is very beneficial for the quality of the end product, which reaches similar quality levels as bags produced with bulk sterilisation, or as liquids sterilisation.

There is no general type of vacuum cycle. The amount of pressure pulses, the height of the pulses, etc. : all parameters depend on the situation. Trial-and-error is the only way to work out an optimally working vacuum sterilisation cycle. These are a few important observations: 1) The pressure increase through steam supply is the most crucial point of the cycle. If this increase is not fast enough, the steam will not be able to penetrate deep into the bags. That means the steam generator must be able to supply sufficient steam and that the piping, valves and filters must be operating perfectly. 2) If the pressure increases too fast, the bags will compress. If the pressure decreases too fast, the bags will explode. 3) The difference between the top and bottom of a pulse must be at least 0.3 bar. 4) When using this type of autoclaving cycle, permanent measurements inside the bags during each cycle are essential. ←