i'Tec_S8
November 2008

Keywords: **Decontamination, Enterobacteria, Method**

Assessment protocol concerning the bacteriological efficacy of an acidulant or any product considered to act on enterobacteria

1. Focus

This type of assessment is designed to determine the efficacy of a single acid, a mix of acids or other product on a feedstuff's enterobacterial load. The treatment will be considered effective if a 3-log reduction in the enterobacteria population is recorded in a given time.

2. Principle

The study principle consists in introducing an active product at a defined dosage into a feedstuff that has been overcontaminated by milling waste. After a given action time, the product's effect is then tested by comparing it against the enterobacteria population in the initial feedstuff containing the flora.

3. Equipment and apparatus

3.1. Raw materials

3.1.1. Milling waste

The feed meals are initially contaminated by mixing in waste from the mill scourer. They generally record a contamination of around 10^7,

3.1.2. Feedstuffs

The effect of the active product is tested on the manufacturer's chosen targeted feedstuff types. The formula and the physical characteristics of the feedstuff(s) are measured or recorded.

3.1.3. Acid or active product

The manufacturer provides the acid or active product, which is incorporated at the manufacturer's specified dosage and incorporation procedure.

3.2. Mixer

A 100-l Théaudin brand blade mixer is used to incorporate the acids and milling waste.

3.3. Spraying apparatus

Liquid acids are sprayed using a system that makes it possible to record an incorporation mass balance. This apparatus consists in a 2-l stainless steel tank and a compressed air network used to pressurise the system (0 to 5 bars). Twelve shallow-angle injection nozzle formats can be used with this system.

3.4. Sampling and packing apparatus

The mixer and receiving tray are cleaned by brushing and vacuuming. Once mixed, the feeds are placed in 20-l non-sterile plastic bags, and then divided up using two splitters of differing size.

The mixes are divided until obtaining samples of approx. 100 g (+/-10g). These samples are then placed in clean, non-sterile plastic pots.

4. Method

4.1. Mixing

The milling waste is incorporated at a rate of 2% for 50 kg of feedstuff. Half of the feedstuff batch is placed in the mixer, the milling waste is then distributed over the whole length and the remaining half of the feedstuff is placed on top. After mixing for 2 minutes at 60 rpm, quartered sub-samples are taken from the mix, prior to adding the acid.

Powdered products are weighed using a +/- 1 g scales and the feedstuff with a +/- 2 g scales. The acid is added and the whole remixed for approx. 2 minutes at 60 rpm. The mix is emptied into the receiving tray, and then immediately placed in 20-l plastic bags using a clean shovel and identified with the mix code.

For liquid product mixes, the acid is incorporated by spraying milling waste over the mix. The spraying process requires a preliminary preparation phase, which consists in weighing the empty spraying system using a +/- 1 g scales, and then filling it with the required quantity of acid (+/- 5g). The spraying system is then placed on the mixer lid and connected to the compressed air supply. The pressure gauge that controls the acid pressure is set at 1.5 bars. The

mix is switched on and spraying starts 10 seconds later. The total homogenisation period is 2 minutes.

The resulting mix is emptied into a receiving tray, and then immediately shovelled into 20-l plastic bags and identified. The total mix weight is determined and recorded on a test record sheet. The mixer is cleaned by scraping with a brush and spatula.

The spraying system is then disconnected from the compressed air network and removed from the mixer lid. The system is reweighed on a +/- 1g scales; the weight is recorded, vent open.

4.2. Preparing samples

4.2.1. Cleaning the equipment

Equipment used for divisions greater than 2 kg (quarter splitter) is cleaned by brushing and vacuuming. The riffle splitter and related moulds are cleaned with soapy water before the tests, and then air-blown between each batch.

4.2.2. Divisions

The whole of the manufactured batch containing the acid is divided to form at least **eight 100-g** representative **samples**. At least **eight 100-g** representative **samples** of the initial feedstuff are also prepared.

4.3. Shipping the samples

The samples are shipped to the analysis lab at the requested temperature(s), and tested within 24 h of mix production.

4.4. Analysis

The initial contamination level is validated after incorporating the milling waste and before mixing in the acidulants. These levels are then checked 24h after incorporating the acidulants.

The samples are therefore analysed **24 h after incorporation**. In all, each test involves at least 16 analyses on **100-g** samples, with each sample undergoing an enterobacteria count. These counts are made according to standard NF V08-054 (37°C), or similar, with a quantification limit of 10 CFU/g for the initial samples, and a limit of 1 CFU/g after the acid has taken effect. The laboratory uses buffer peptone water and checks that the initial suspension is pH neutral. The presence of non-acidulants would mean having to use a product to neutralise the action of the tested product. Where necessary, preliminary tests can be carried out in order to define the neutralisation conditions for the product used.

4.5. Processing the results

The tests are characterised by two types of result:
Test conditions (quantity of acid incorporated, action time, storage temperature, etc.)
Microbiological results

The results of the enterobacteria counts will be interpreted based on:
initial contamination rate
variation in contamination rate
final contamination rate.

It is also recommended to run nonparametric LSD tests.