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Technical rules for assessing the bacteriolytic effectiveness of a pelleting line or a heat treatment

The implementation of European regulations 183/2005 of 12/01/05 that establishes feed hygiene requirements, and 2160/2003 of 17/11/03 on control of salmonella in the food chain led to the publication in the French Official Journal of the Order of 23 April 2007 relating to approvals of business operators in the animal feed sector. This order introduced a "Salmonella" approval, which, in the very near future, will become mandatory for all feed operators that deliver to establishments that rear breeding laying hens. Annex IV of this order states that "All compound feedstuffs distributed to *Gallus gallus* breeding hens shall be treated by a validated process that guarantees a minimum 3-log reduction in microbial contamination in the form of enterobacterial infection". Feedstuffs produced following this treatment shall contain no more than 1000 enterobacteria per gram.

Tecaliman has conducted a research programme funded by the following organisations - Office de l'élevage, Onidol, ONIC, Dgal, Inaporc, Cidef, CIP, and Tecaliman - aimed at validating that, under certain conditions, these decontamination requirements can be met by the pelleting process.

This programme revealed that there are 4 types of feedstuff that can be pelleted under conditions that ensure a 3-log reduction in the enterobacteria population. It also identified indicative pelleting parameters that can be fine-tuned at each industrial site to achieve the same level of reduction.

Plants will then be able to verify the effectiveness of the selected conditions using the protocol set out in this document. Although the programme targeted the pelleting process, the protocol can also be used to assess the effectiveness of any type of heat treatment.

1. Focus

These technical rules are designed to assess the bacteriolytic effectiveness of a heat treatment line under specific treatment conditions that exist between the times the product enters the treatment line and exits the cooler.

The aim is to demonstrate that, under test conditions, the treatment line is capable of producing:

- a 3-log reduction in the feedstuff's enterobacteria population
- an enterobacteria contamination rate at the cooler output of less than 1000 enterobacteria/g of feedstuff (Order of 23 April 2007).

2. Principle

The method consists in:

- Selecting a treatment line
- Selecting a feed type that will be used to produce a batch
- Defining the treatment conditions (temperature, treatment flow rates or times, etc.)
- Taking sterile samples pre and post-treatment (cooler output)
- Treating the samples and counting the enterobacteria on representative test portions
- Processing and interpreting the results

3. Equipment and apparatus

3.1. Tracer

The tracer consists in the enterobacteria naturally present in the chosen feedstuff. Samples are not screened for salmonella due to its low prevalence and the absence of a standardised counting process.

3.2. The batch

The batch consists of a given feedstuff, and may comprise one or several mixer loads provided that this constitutes the overall "treated batch". Due to the variability in results according to feed type (residence time distribution, effect of feed composition on heating, etc.), the treatment conditions will only be validated for the selected feed type.

3.3. The line

The line has to be properly characterised and identified at the time of the tests. This will involve noting the date of the tests, the type of equipment and apparatus found between the two end sampling points and, for a new test on a previously validated line, details of any changes to or work carried out on the line between the two tests. Any cleaning or disinfection work that has been carried out on the line recently will also have to be noted.

For pelleting lines, special attention should be paid to

the die:

- Condition
- Age
- Number of holes and their diameter
- Compression length (deduct the length of the counterbore)

3.4. Measurements

3.4.1. Line

3.4.1.1 Temperature

The temperature probes fitted on the line must be checked and validated. In terms of their general day-to-day use, these probes have to be cleaned regularly and placed in the product flow (subscribers to Tecaliman can refer to Technical Datasheet No. 26).

3.4.1.2 Steam

The steam pressure has to be verified and recorded.

3.4.2. Flow

The in-production instantaneous flow rate has to be determined as accurately as possible. This should be estimated by deducting the loading periods, and estimating the proportion of fines and their recycling period. The size of the margin applied to the chosen scale depends on how accurately this flow rate is estimated, i.e. the lesser the accuracy, the greater the margin.

3.4.3. Test management

3.4.3.1 Time

Samples are taken at specific times, which are determined prior to the start of batch pelleting. This requires two stopwatches. The first is used by the operator at the press output to note their sampling times. The second is used by the operator at the cooler output.

3.4.3.2 Temperature

The temperature of the feed - pelleted or not - can be measured at any sampling station using an adiabatic vessel such as a Thermos and a mobile temperature probe.
Current state-of-the-art means it is very difficult to take in-line measurements of pellet temperature at the press output. Although the technique using a Thermos and a probe is practical, it only provides a snapshot of pellet temperature, and may be slightly skewed.

3.4.3.3 Residence times

Determining die residence times for pelleted feeds requires detailed data on die characteristics. The estimated mass of 1 cm of pellets is measured using a graduated glass rod into which the pellets are placed. Next, the mass of 20 cm of pellets is measured.
On non-pelleting lines, residence times can be estimated, and this estimated value supplemented by an assessment of residence time distributions (subscribers to Tecaliman can refer to Technical Datasheet Nos 53 to 56).

3.5. Sampling

3.5.1. Sampling equipment

Samples are taken with metal shovels and then placed in sterile Secure-T Stomacher bags with wire mesh closures.

3.5.2. Disinfection equipment

All equipment that is in contact with the samples has to be disinfected.
A heat gun can be used to dry/ disinfect the equipment in order to remove all traces of liquid disinfectant that could decontaminate the feed directly, taking care with the effect of high temperatures on the materials used in the sampling procedure.

3.6. Processing the samples

Samples are packed before being sent to the lab. This step requires the use of riffle splitters, and can be performed by the laboratory if it has the necessary equipment and methods. It is possible to analyse all the samples, but this would increase test costs.

3.7. Storage and Transport

During testing days, the samples are stored:

- in a refrigerator, if the plant has easily accessible equipment,
- in refrigerated insulated boxes (option of using frozen eutectic packs). There must be no direct contact between the cold source and the samples.

Samples are stored in positive cold conditions (+ 4°C), and sent to the laboratory in insulated boxes containing an internal cooling system. It is recommended to place a "chronotemps" indicator inside the boxes with each delivery in order to confirm that the temperature has never exceeded the level that would allow bacterial growth (above +4°C).

4. Method

4.1. Batch size

Treated batches should enable an output of at least thirty minutes at rated speed in order to ensure that all the necessary samples can be taken at the press and cooler outputs.

4.2. Batch number

A treated batch may correspond to more than one mixer load.

4.3. Sampling

4.3.1. Sampling point

The initial contamination is determined by taking samples at the mixer or molasser outputs. If the "treated batch" is made up of several loads, the increments will be distributed over the loads as a whole.
Post-treatment contamination is determined by

analysing the samples taken at the cooler output.
Samples may also be taken at the heat treatment output if the study solely concerns decontamination on the treatment line without taking account of recontamination risks. In this case, the samples will have to be dried and cooled individually outside the production circuit. Every possible precaution to protect against recontamination must be taken during this cooling process.

4.3.2. Sampling procedure

The equipment used to take and treat the samples must be disinfected and dried prior to use (a few seconds with the heat gun will suffice). Samples are taken in a product flow, taking care to vary the direction when cutting the flow at each increment. Care should also be taken to avoid soiling the samples with deposits in the area of the sampling point.

4.3.3. Sample number and size

To obtain satisfactory batch representativeness, at least four 50-g increments are taken at each studied sampling station. Tests carried out by Tecaliman have revealed that enterobacteria contamination is fairly uniform and that 4 samples is sufficient to provide an aggregate sample of approx. 200g that is representative of the batch, with a confidence rate of at least 95%.

4.3.4. Frequency

Sampling frequency is determined to ensure the samples are spread over all the mixed loads or over the treated batch during an output at rated speed. This means that the throughput time (flow rate) needs to be estimated at each sampling station prior to running the tests.
At each sampling point, the first increment is always sampled after a given time period, and there should be an equivalent time period after taking the last increment.
Increments are time-shifted if samples are taken upstream and downstream of the cooler. If vertical coolers are used, the samples are taken at closer intervals and have to coincide with emptying cycles. Pellets from the first and last cycles should not be sampled.

4.3.5. Other sampling operations

One-off samples can be taken at each sampling station in order to obtain data used in interpreting the results:

- Initial physical properties of the feed
- Moisture content
- Durability/hardness/proportion of fines

4.4. Testing

4.4.1. Batch follow through in the manufacturing circuit

Preferably, the tests should not be performed on a line that has been used to treat medicated feed or any product likely to impact on bacterial growth. If this is the case though, at least three batches free of such products will have to be produced in the same silo bin upstream of the press before conducting the test in order to minimise the effect of carry-over.

4.4.2. Measurements

For samples taken at the treatment output, the product temperature at this sampling station can be measured using a portable probe and an adiabatic vessel, such as a "Thermos".

4.4.3. Data to be recorded

In order to interpret the results and determine accurate test conditions, the following data has to be recorded:

- Batch size (weighing record(s))
- Overall batch flow rate
- Instantaneous batch flow rate in rated output (durations of the power build-up, rated output and recycling phases) - this may be done by recording the press power demand. If indicated by the PLC, the method used to assess flow rate should also be considered.
- Controlled treatment temperature
- Steam pressure
- Setpoint temperature (any records on the line could be collected)
- Die characteristics
- Nature of the 3 batches produced on the line prior to testing
- Press strength in rated operation and any fluctuations

4.5. Sample processing and analysis

Increments sampled at a given sampling point must be grouped and split up under aseptic conditions. Aliquots are packed under clean conditions insofar as repacking is arranged in increasing order of theoretical contamination.
If sample splitting and analysis is performed at different locations, one portion is held in reserve in positive cold conditions (+4°C) at the splitting location. This ensures the availability of duplicate samples should storage conditions deteriorate during transportation to the testing lab. The samples must be shipped within 24 h under conditions that ensure their preservation (positive cold).

4.6. Analyses

Each sample undergoes an enterobacteria count. These counts are made according to standard NF V08-054 (37°C) with a quantification limit of 10 CFU/g for samples at the mixer output, and with a limit of 1 CFU/g for other samples. In the months ahead, this standard will require an amendment concerning standards ISO 21528-1 and 2.

If disinfectants are used in the process prior to the tests, or if there is a chance of the samples containing traces of antibiotics, the laboratory should use an appropriate neutralizer for the product in question.

4.7. Expression and interpretation of the results

The tests are characterised by two types of result:

- Implementation conditions
- Microbiological results

4.7.1. Implementation conditions

This means characterising the conditions under which the decontamination results were obtained, so as then to be able to demonstrate that the treatments are performed systematically under these conditions (control conditions), to define the tolerance limits for these conditions and plan which corrective measures to deploy to counter any loss of control.
The principal conditions involve:

- Treated feedstuff
- Treatment temperature
- Residence times

Die residence times for pelleted feeds are determined (in seconds) according to the following equations.

- Residence time = Mass of the pellets in the die (kg)/ Instantaneous flow rate (kg/s).
- Mass of pellets in the die = Mass of 1 cm of pellets x Number of cm in the die
- Number of cm of pellets in the die = Channel compression length in cm x Number of channels

While the residence time in the press conditioner may impact on the microbial flora, this appears to be less significant in conventional pelleting lines than in the die.
Note that where heat treatments are concerned, while the flow rate is an item, it is essential to measure residence time distributions.

Test conditions are therefore also determined on the basis of records established during the testing phase:

- Date of the test
- Overall batch flow rate
- Instantaneous batch flow rate during rated output
- Packing temperature
- Steam pressure
- Die characteristics
- Type of feedstuff (formula)
- Initial physical characteristics of the feedstuff (particle size, density, moisture content, etc.)
- Mass of 1 cm of pellets

Additional parameters can also be used to interpret the results and to plan corrective or preventive measures:

- Temperature at the treatment output if this differs from the packing temperature
- Durability
- Moisture content at every sampling point

4.7.2. Microbiological resultsThe results of enterobacteria counts will be interpreted based on:

- initial contamination rate
- minimum 3-log variation in contamination rate between the time the product enters the treatment process and the time it exits the cooler.
- final contamination rate, which must be less than 1000 CFU/g at the cooler output.

Several, non-exclusive, causes may be considered in the event of inadequate decontamination:

- Exceptionally high initial contamination of the feedstuff (which may differ from individual material contamination rates)
- Significant recontamination between the treatment location and the sample or batch sampling point
- Sample preparation/transportation conditions that diminished their representativeness
- An inadequate treatment scale

5. Conclusion

These tests must be carried out as part of an HACCP process (subscribers to Tecaliman can refer to i'Tec_S2). The results are incorporated into the heat treatment management process as a critical control point (CCP1) aimed at achieving a specified level of microbiological quality. After completing the tests on a target line, the treatment must be managed as part of a CCP procedure; it should be possible to demonstrate control of the treatment procedure under test conditions at any time (residence time, flow rate, temperature, etc.). It will no longer be possible to treat a batch below the setpoints established for a given line without risking downstream line contamination. If the line is contaminated, procedures will have to be implemented following the passage of the contaminated batches, in order to limit the risk of recontamination downstream of the circuit. Preventive and corrective measures must be planned and set up as part of this framework. The concept of targeted microbiological quality diverges from current industry pelleting practice, meaning that its successful deployment requires raising awareness among feedstuff production operators. It also means having to manage risks of recontamination downstream of the treatment process (CCP2) and up to loading into lorries, as this item is specified in the Order of 23 April 2007 (subscribers to Tecaliman can refer to i'Doc_S11 and i'Tec_S5).

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