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QUALITY CONTROL METHOD - 53

Ammonia Oxidation Rate Measurement

Description:
This quality control procedure is designed to measure the ammonia oxidation rate of Nitrosomonas europaea (CF 1100-50xFF/7110-50xFF manufacturing concentrate) in the production reactor or in the laboratory, immediately after manufacturing is completed. Dilutions are made from this concentrate to meet individual product specifications. For finished formulas, perform QC-2: STP test to verify survival of autotrophic nitrifier cultures. This procedure should be performed by a trained laboratory technician.

1. Equipment:

1.1 1.5 liter LH fermenter glass vessel or equivalent
1.2 Magnetic Stirrer with moderate size stir bar
1.3 Vacuum flask
1.4 Temperature probe
1.5 Submersible heater with temperature control
1.6 pH Controller with probe
1.7 Air stone
1.8 Masterflex® silicone-platinum (autoclavable) Tubing
1.9 250 ml Pyrex pressure-equalizing separatory funnel for K_2CO_3 (used as addition vessel)
1.10 Automatic Addition pump, adjusted to 0.8 to 1.0 rpm
1.11 Regular laboratory analytical balance
1.12 Denver Scale - computer driven analytical balance with settings adjusted as follows:

Units = grams
Doors = manual
Autocalibrate = off
Autozero = off
Filter = 3
Beeper = off
Print interval = 60 seconds
Print = continuous, after each update
Zero Print = off
Output format = 6
Baud = 9600
Parity = None
Echo = On

1.13 Computer with spreadsheet program set to calculate the following:
- 1.13.1 Slope of the line for K_2CO_3 consumption in mLs/min from the linear portion of the line.
- 1.13.2 Calculate the NH_3-N/liter/hour, updated automatically every minute using the following calculation:
  - 1.13.2.1 (K_2CO_3 rate mls/min) x (60 min/hr) x (50000 mg K_2CO_3/1000 mls) x (dilution)
- 1.13.3 Calculate the slope of the line in mg NH_3-N/minute and ammonia oxidation rate for ammonia utilization in mg NH_3-N/liter/hour using the following calculation:
  - 1.13.3.1 (NH_3-N ppm/minute) x (60 min/hr) x (dilution)

1.14 Dot Matrix printer loaded with continuous-feed graph paper.
1.15 Autoclave
1.16 Nalgene MF 75 1 liter filter sterilizing units with 0.2 µm pores (stocked by the case)
1.17 CHEMetrics visual test kits
- 1.17.1 K-1510 and 1510D to test ammonia-nitrogen, APHA Standard Methods 4500 NH3 C
- 1.17.2 K-7002B and/or 7002C to test for nitrite-nitrogen, APHA Standard Methods 4500.NO2B

2. Ingredients:

2.1	Unbuffered Nitrosomonas Medium (see below)	100 mls
2.2	Solution B (see below)	100 mls
2.3	Solution C (see below)	100 mls
2.4	Ammonium chloride solution	30 mls
2.5	Deionized water	5 Liters
2.6	Sodium hydroxide - fresh solution (20 ml 10N NaOH in 800 ml deionized water)	820 mls
2.7	5% K_2CO_3 solution (prepared monthly)	200 mls

Unbuffered Nitrosomonas Medium (Autoclave solution for 15 minutes at 121° C or use Nalgene MF 75 - 1 Liter Filter Sterilizing units with 0.2 µm pores to filter-sterilize,)

1.	$MgSO_4 \cdot 7H_2O$	0.7 g
2.	$CaCl_2 \cdot 2H_2O$	0.02 g
3.	Solution B	1 ml
4.	Solution C	1 ml
5.	Deionized water sufficient to make	1 Liter

Solution B (store refrigerated after filter sterilizing through 1 Liter Nalgene MF75 Filter Sterilizing Unit with 0.2 µm pores) **Identical to "QC 2" - Solution B.** Store refrigerated for up to 1 year

1.	Sequestrene® or *Sigma EDTA - NaFe* (12% iron) (EDTA NaFe)	1 g
2.	Distilled water	1 L

Solution C (store refrigerated after filter sterilizing in 125 ml Nalgene MF75 Filter Sterilizing Unit with 0.2 µm pores)

1.	100 mg $Na_2MoO_4 \cdot 2H_2O$/100 mls	10 mls
2.	100 mg $MnSO_4 \cdot H_2O$/100 mls	17.2 mls
3.	100 mg $CoCl_2 \cdot 6H_2O$/100 mls	0.4 mls
4.	100 mg $ZnSO_4 \cdot 7H_2O$/100 mls	10 mls
5.	Deionized water sufficient to make	100 mls

3. Procedure:

3.1 Add appropriate amount of nitrifiers to LH fermenter glass vessel, or equivalent.

3.1.1 Adjust amount to be added based on potential activity (10 to 875 mls). The reaction can be slowed by too few or too many cells. If too many cells are present, the amount of dissolved oxygen will be insufficient. If not enough cells are present, the oxidation rate will be very slow and the change in ammonia concentration will require extended testing.

3.1.2 Add 100 mls unbuffered Nitrosomonas medium (see above) to replenish any necessary minerals that may have been washed out during ultrafiltration.

3.1.3 Add 30 mls 5% (weight/volume) ammonium chloride solution.

3.1.4 Bring to one Liter with deionized water.

3.2 Agitate with vigorous stirring, usually a setting of about 7 is needed on the magnetic stirrer with a moderate size stir bar.

3.3 Aerate vigorously with CO_2 free air and an air stone.

3.3.1 Using an air stone, vigorously bubble air through a solution of freshly prepared sodium hydroxide (20 ml 10N NaOH in 800 mls deionized water) in a vacuum flask, to produce air that is free of carbon dioxide.

3.3.1.1 The flow of air should be as follows: air supply → flow meter → top of NaOH vacuum flask → side port of NaOH vacuum flask → sample vessel.

3.4 Insert temperature probe and heater into vessel, then turn on temperature control and allow reactor to reach equilibrium at 30°±0.3°C. Do not operate the heater without submerging it in liquid first.

3.5 Turn on the pH controller and calibrate pH probe. Record calibration on log sheet.

3.6 Check calibration of the Denver Scale computer driven analytical balance and record on log sheet. Manually calibrate if needed. Confirm setup of this balance, as listed under equipment, above.

3.7 Tare Denver Scale with empty 250 ml pressure-equalizing separatory funnel on it.

3.8 Fill 250 ml pressure-equalizing separatory funnel with 5% K_2CO_3 solution (prepared monthly) and place on scale.

3.9 Prime the addition tubing with K_2CO_3 solution.

3.10 Adjust pH to 8.0 with 5% K_2CO_3 solution. There should be no air in the K_2CO_3 solution tubing after this adjustment. Caution: Adjust pH gradually in case sample has negligible buffering capacity.

3.10.1 Adjust addition pump rpm's (typically 0.8 to 1.0).

3.10.2 Make sure the pH control switch on the back of the unit is set to "auto" and not "manual".

3.11 Allow the temperature and other parameters to stabilize.

3.12 Turn on the computer and data acquisition and begin timing the assay.

3.12.1 Turn on the computer, monitor and printer.

3.12.2 Double click on "Collect" short-cut icon.

3.12.3 Fill in the necessary information between data logger updates (lot, date, initials, sample volume, etc.) Note: Rate will not calculate without the sample volume used.

3.12.4 Set the Data Acquisition (Collect/W) Parameters as follows:

3.12.4.1 Port = Com 1, Baud = 9600, Parity = None, Data Bits = 7, Stop Bits = 1, Protocol = None, Time Out = 250

3.13 Monitor assay periodically, to record information on log sheet, as needed.

3.13.1 Check pH, temperature, airflow, rpm setting (usually 7) and pump setting (usually 0.8 to 1.0) to make sure that everything is functioning properly. Check for clamps, leaks, etc.

3.13.2 Measure ammonia-nitrogen concentration periodically, using appropriate CHEMetrics visual test kit and record on the log sheet and in the spreadsheet.

3.13.3 If the activity is poor, it is not unusual for the pH to increase for awhile before it begins to decrease. If this happens, periodically adjust pH manually with acid to 8.0 until activity starts.

3.14 Stop the assay four hours after activity is detected. This is an arbitrary time point chosen because K_2CO_3 consumption rate generally increases as the test progresses. The lag phase varies based on the activity and age of the product tested. Wait for a maximum of 24 hours for activity to begin.

3.14.1 The spreadsheet will automatically calculate the slope of the line for K_2CO_3 consumption in mls/minute from the linear portion of the line. The ammonia oxidation rate in mg NH_3-N/liter/hour is predicted from the K_2CO_3 rate and will also update automatically every minute using the calculation listed under “Equipment - 1.13.2.1” above. Delete unnecessary K_2CO_3 values from the lag phase before determining the final rate.

3.14.2 The spreadsheet will automatically calculate the slope of the line in mg NH_3-N/minute/hour using the second calculation listed under “Equipment - 1.13.3.1” above.

3.14.3 Average both rates to determine the final ammonia oxidation rate.

3.15 Print out graphs. Store paperwork in binder for a minimum of 3 years. Record final results in appropriate computer QC database.

3.16 Retest concentrate monthly to verify correct dilutions for finished products.