A novel anammox bacteria, Candidatus Brocadia caroliniensis, having Accession Deposit Number NRRL B-50286 and the characteristics of oxidizing ammonia and releasing di-nitrogen under anaerobic conditions, has been discovered. It can be used for effective treatment of wastewater having undesirable levels of ammonia, including agricultural, industrial, or municipal wastewaters. The obtained anammox sludge has been used for quick start-up of bio-reactors using the deammonification process.

Deammonification process: Partial nitratation + anaerobic ammonium oxidation (anammmox)

CONCEPT
- Totally autotrophic bioprocess for the removal of nitrogen

TARGET
- Low cost treatment of wastewaters containing high ammonia nitrogen

ADVANTAGES (relative to conventional nitrification-denitrification)
- Reduces 58% of the oxygen requirements and 83% of biosolids production
- Reduces 100% of the carbon needs for denitrification
- Does not produce nitrous oxide, a powerful greenhouse gas
- Nitrogen removal rates are five to ten times higher

Isolation of anammox
- Anammox bacteria were isolated from livestock manure sludge using synthetic media. PVA porous beads were used for biomass attachment (Exp 1 & 2)
- A pilot reactor that used non-woven carrier was inoculated with the isolated anammox; it showed quick start-up and high activity (Exp 3)

Up-flow pilot reactor using non-woven carrier

N Removal Rates in Pilot Reactor Seeded with Anammox Sludge (Exp. 3)

Immobilized anammox pellets
- Mix of anammox and PVA was polymerized with the freezing technique. Pellets were used in continuous stirred reactors (CSTR) at 20% v/v
- The immobilized anammox recovered 81% of the potential activity

Anammox coupled with partial nitratation (two-stage process)
- Used two sequencing batch reactors (SBR), one for partial nitratation (PN) and the other for anammox reaction to treat swine wastewater with 1400 mg NH$_4$-N L$^{-1}$
- The PN used suspended High Performance Nitrifying Sludge (HPNS) (NRRL B-50298) and produced an effluent with a NO$_3$-N:NH$_4$-N ratio of 1.4
- The anammox reactor used suspended granular Brocadia caroliniensis (NRRL B-50286). Influent NO$_3$-N + NH$_4$-N was removed at rates up to 1,340 mg/L-reactor/day.

Single tank N removal using anammox and partial nitratation (one-stage process)
- Used one continuous flow aerated reactor containing suspended nitrifying sludge (NRRL B-50298) and suspended anammox Brocadia caroliniensis (NRRL B-50286).
- The reactor contained biofilm plastic carriers (1200 m$^2$/m$^3$) at 30% v/v packing ratio that were fluidized by the aeration.
- The water temperature was 22°C and DO <0.3 mg L$^{-1}$
- Influent contained 200 mg NH$_4$-N L$^{-1}$ and 1500 mg carbon alkalinity L$^{-1}$
- Total N removal rates of 750 to 1000 mg/L were obtained during the first 3 months
- The stoichiometry of the reaction obtained (batch test) was consistent with deammonification process.

Deammonification stoichiometry theory:
1. Partial nitratation:
   - 2 NH$_4$+ + 1.5 O$_2$ $\rightarrow$ NH$_4$+ + NO$_2$ + H$_2$O + 2 H$^+$
2. Anammox:
   - NH$_4$+ + 1.32 NO$_2$ $\rightarrow$ 1.02 N$_2$ + 0.26 NO$_3$ + 2 H$_2$O

Single-stage stoichiometry (data shown below):
   - NH$_4$+ + 0.85 O$_2$ $\rightarrow$ 0.44 N$_2$ + 0.11 NO$_3$ + 1.43 H$_2$O + 1.14 H$^+$
   - NH$_4$+ + 0.88 O$_2$ $\rightarrow$ 0.44 N$_2$ + 0.11 NO$_3$ + 1.43 H$_2$O + 1.14 H$^+$

Anammox bacteria characterization
- DNA analyses indicated novel species of anammox in the USA
- Candidatus “Brocadia caroliniensis”
- Isolated from livestock manure sludge
- Specific molecular probe FLO1 and Fish protocol developed

Stoichiometry and treatment performance
- Average nitrogen removal rate obtained during 1 year at HRT of 3.9 hours was 1477 mg N/L-reactor/day
- Stoichiometry of Brocadia caroliniensis was determined under wide range of N loading rates during 3.8 yr period

N removal rates in continuous up-flow pilot reactor

Stoichiometric coefficients of anammox

Long-term anammox preservation
- Long-term preservation was needed to deposit Brocadia caroliniensis in international depositary authority under Budapest Treaty of United Nations (WIPO)
- A preservation protocol was successfully developed using lyophilization after freezing with skim milk at -20°C
- This protocol was used to deposit Brocadia caroliniensis under Budapest Treaty provisions in the Agriculture Research Culture Collection in Peoria, IL, under Accession Deposit NRRL B-50286.