Subject: **Testing of [redacted] denitrification substrates for municipal wastewater treatment plant activated sludge.**

[redacted]

Methodology of denitrification test under anoxic conditions

Generally, the main result of denitrification test is a value of denitrification rate, i.e. rate of nitrate nitrogen reduction into gaseous nitrogen through a biochemical process. The process rate is expressed in two distinct forms:

- **volume rate** – a rate expressed in units of volume: mg/(L h) or g/(m^3 h).
- **specific rate** – a rate per unit of dry sludge – mg/(g h) –, respectively an organic fraction of dry sludge showing which biomass fraction is active and which one is inert.

The tests are carried out under anoxic conditions (without molecular oxygen); the measured value is nitrate nitrogen loss in time and information on denitrification rate of activated sludge is obtained. Tested sludge is aerated for several hours to come into a state of endogenous respiration. The oxygen supply is then switch off and oxygen concentration is left to drop to zero (can be speeded up by nitrogen bubbling). The reaction vessel must be sealed. For this purpose, Erlenmayer flask is a suitable reactor, for example. The reaction temperature has to be kept at constant level throughout the test. For anoxic tests, the S_0/X_0 ratio is used to be 0.05–0.1. Throughout the test, sufficient concentration of ammonia nitrogen for synthesis (5–10 mg/L) and orthophosphate phosphorus (more than 1 mg/L) has to be ensured in the mixed liquor. Before mixing organic substrate and sludge, nitrate nitrogen is dosed into the mixed liquor; the dose shall be such that its initial concentration ranges from 50 to 100 mg/L after mixing. The first sample is taken before mixing. It is very important to process samples immediately, i.e. to filtrate them through a membrane filter, possibly after prior centrifugation. Then the sludge and treated substrate are mixed and samples are being taken at regular intervals for 2 to 3 hours. The samples are analysed for nitrate nitrogen concentration.

Denitrification rates can be deducted from measured curves and in the case of breakpoint, a substrate portion with regards of its utilization at the highest, i.e. the maximum denitrification rate can be determined.