Pilot-scale trial for the removal of nitrogen from geothermal water using Lentikats Biotechnology

Final report

December 2010

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Table of content

1. PROJECT DESCIPTION

The aim of the project was to verify the applicability of Lentikats Biotechnology for nitrogen removal from geothermal water. The here presented report summarises the main findings of a pilot-scale trial carried out at Tikitere from September 2010 to December 2010. The project was carried out as a part of the Lakes Rotorua restoration project managed by Bay of Plenty Regional Council.

2. BACKGROUND

2.1. Geothermal water

The geothermal streams in Tikitere area have been identified as one of the main sources of nitrogen pollution for Lake Rotorua. The geothermal streams are contributing about 30 tonnes of nitrogen per year to the lake with their average daily flow of 4400 m^3/day (Vega *et. al* 2007). Previously completed projects suggested on-site treatment of the streams as the most viable option for elimination of this source.

The geothermal water at Tikitere area is characterised by a very low pH (~3.0), high temperatures (~28°C) and elevated concentrations of heavy metals. Despite these somewhat extreme characteristics, previous test (Vega *et. al* 2007) indicated a possible treatability of this water by biological methods, i.e. ruled out any inhibiting effect of this water on activated sludge and its nitrification activity.

2.2. Lentikats Biotechnology

Lentikats Biotechnology is a process based on immobilisation of bacteria or free enzymes in porous matrix made of polyvinyl alcohol (PVA). The immobilisation ensures a presence of high concentration of pure bacterial culture, thus creating highly efficient and selective solution. The PVA matrix possesses excellent physical-chemical properties; it is biologically non-degradable and has zero toxicity. Application of such Biocatalyst will have no side effects on the main process.

For removal of inorganic forms of nitrogen from aqueous solutions (wastewater, drinking water), LentiKat's has developed two Lentikats Biocatalysts:

- **nitrification Biocatalyst** – containing high concentration of nitrification bacteria (*Nitrosomonas europaea, Nitrobacter winogradskyi)*
- **denitrification Biocatalyst** – containing high concentration of denitrification bacteria *(Paracoccus denitrificans)*

In comparison to other available technologies, Lentikats Biotechnology presents a stable, compact and reliable alternative, providing 98% removal efficiency. Presence of high concentration of the immobilised bacteria results in high removal rates, which consequently allows for shortening of required retention time and reduction of required reaction volumes. With respect to the above, Lentikats Biotechnology leads to operational as well as investment cost savings.

It has been previously shown that the immobilisation of nitrification and denitrification bacteria using Lentikats Biotechnology enhances their robustness toward negative environmental effects (Bouškova *et. al* 2009; Trögl *et. al* 2010), such as a presence of potentially inhibiting substances or high salinity. Based on these promising results, Lentikats Biotechnology has been selected as a viable option for the treatment of geothermal water.

3. PILOT-SCALE TRIAL

3.1. Geothermal water

The pilot-scale trial was carried out using authentic geothermal water at the Tikitere geothermal field. The main characteristics of the water are summarised in Table 1.

Table 1: Main characteristics of geothermal water

Parameter	Average value	Unit
pH	3.2	-
$N\text{-}NH_4^+$	30.7	mg/L
temperature	24.6	℃
TDS	567.5	mg/L
Conductivity	985.0	µS/cm
Cl^-	6.9	mg/L
SO_4^{2-}	230	mg/L
S^{2-}	0.37	mg/L
Hg	0.0096	mg/L
Pb	0.0022	mg/L
Li	0.0123	mg/L
Be	0.00012	mg/L
Mo	< 0.0003	mg/L
Sn	< 0.00053	mg/L
CN^-	< 0.0010	mg/L

3.2. Testing unit

The pilot-scale trial was carried out using a container test unit assembled by SCION, the test unit's diagram is presented in Appendix 1.

The treatment system consisted of three reactors with a total volume of 1000 L each, connected in series. The geothermal water was pumped from the stream into the first, pre-treatment reactor. This tank was continuously mixed using a water pump. The pH of the geothermal water was adjusted to ~ 7.0 in this tank by means of controlled dosing of 30% solution of caustic soda (NaOH) from a storage tank based on the response of .a pH probe located inside the tank.

The pre-treated water was pumped using a mono progressing cavity pump into the nitrification tank at the flow rate of about1.5 L/min. The nitrification tank was loaded with 100 kg of nitrification Lentikats Biocatalyst. Four fine bubble air diffusers were mounted to the bottom of the tank, each passing 7.75 m3 of air per hour. The DO, pH and temperature was monitored using online probes. The pH in this tank was adjusted by controlled dosing of 30% NaOH solution based on the response of another pH probe. A specifically manufactured sieve separator was placed to the outlet of the tank to hold the Biocatalyst inside the tank, while the treated water was flowing through the tank.

The pre-nitrified water overflowed from the nitrification into denitrification tank by gravity. 100 kg of denitrification Lentikats Biocatalyst was placed into the denitrification tank at the beginning of the trial. This tank was continuously mixed using two overhead mixers and was equipped with an identical sieve separator as the nitrification reactor. Continuous dosing of 30% ethanol solution into this tank served as a carbon source for the denitrification bacteria. Temperature and pH was monitored using an online probe.

The treated water overflowed by gravity from the denitrification tank into the outlet and was discharged back into the stream.

3.3. Operation

At the beginning of the trial, the nitrification and denitrification Lentikats Biocatalysts were loaded into the respective reactors. Due to the long transportation during which the bacterial activity was suppressed by continuous cooling of the Biocatalyst to 4-10°C, it was necessary to re-activate the Biocatalyst in a series of batch tests using a standard mineral media.

The system was then operated in a batch mode using the real pre-treated geothermal water for another week (7 batches) in order for the Biocatalyst to acclimatise to the specific characteristics of the water. The pre-treated geothermal water was manually transferred from the pre-treatment tank into the nitrification tank within 30 minutes, displacing the nitrified water in the nitrification tank by the raw geothermal water. The nitrified water simultaneously overflowed into the denitrification tank.

Once the Biocatalysts reached a steady-state and a constant activity, the system was switched into a continuous mode with the flow rate of about 1.5 L/min.

Samples from all three reactors were taken regularly during the operation of the trial and analysed for the following parameters: $N\text{-}NH_4$, $N\text{-}NO_3$, $N\text{-}NO_2$ and COD by the Environmental Laboratory of Rotorua District Council. A portable nitrogen test kit RQ Flex (Merck Co.) was used for instantaneous analysis on site.

The activity of Lentikats Biocatalyst is expressed in mg of nitrogen equivalents removed per hour by one kilogram of Biocatalyst.

4. RESULTS

Table 2 summarises the course of the trial. After successful re-activation and adaptation of the Biocatalyst to the geothermal water, the system was switched to continuous operation on October 3. Unfortunately, on October 26 an error intervention of contractors working on a parallel trial caused a long-term shutdown of the main pump. As a result, the water level in the pre-treatment tank dropped below the reach of the pH probe and caustic soda solution was dosed uncontrollably into this tank. The pre-treated water entering the nitrification reached the pH level of 13. Due to the fact that the nitrification pH probe was placed in the centre of the tank causing a certain delay of the error signal to the PLC, the nitrification Biocatalyst was exposed to the pH > 10, which is inhibiting to any bacteria, causing cell lyses. This high pH water had to be flushed out from the system, which took several hours. Consequently, the exposure of the Biocatalyst to this high pH levels lasted for more than 24 hours, resulting in an almost complete deactivation of the nitrification Biocatalyst.

After the replacement of the high pH medium in the nitrification tank by fresh geothermal water with neutral pH, the Biocatalyst showed a minimum activity. On November 10 a new series of reactivation batch test was commenced with the aim to re-cultivate the Biocatalyst, i.e. to re-grow new bacterial population inside the Biocatalysts. After a partially successful re-cultivation (see activity data below), the system was switched again into the continuous mode. The trial was terminated on December 10, when the test unit had to be returned to SCION for other projects.

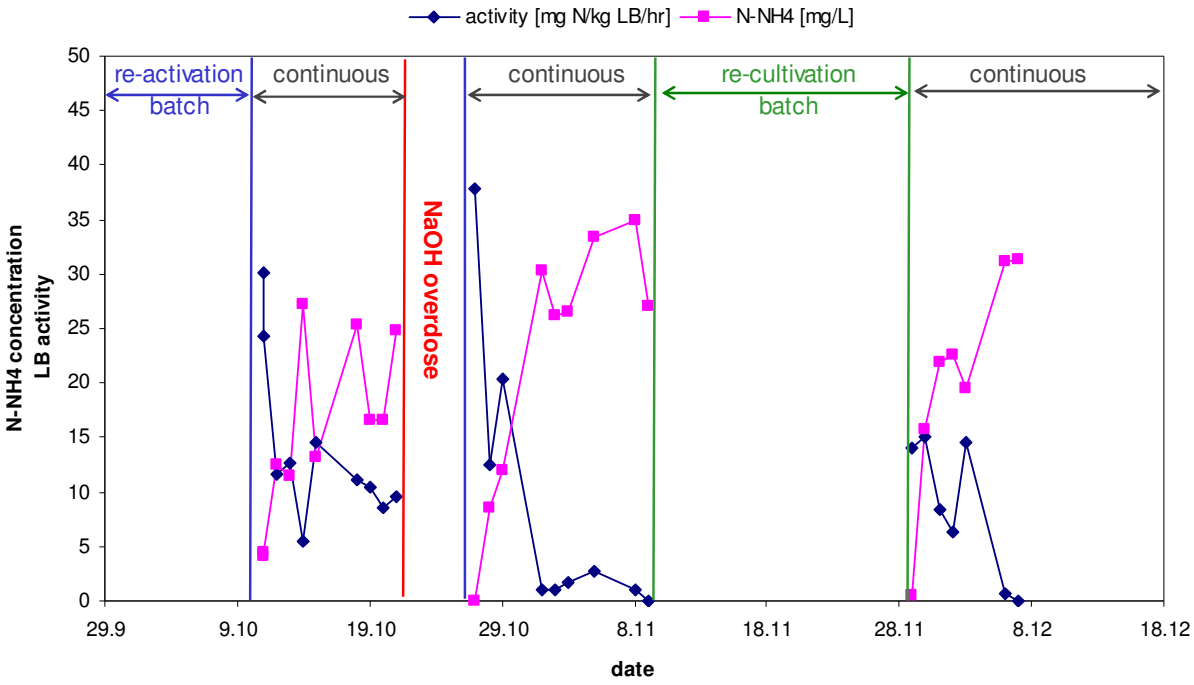
Table 2: Course of the trial operation.

Period	Operation mode	Medium
28.9. – 3.10.2010	Batch	Mineral medium
3.10. – 10.10.2010	Batch	Geothermal water
11.10. – 9.11.2010	Continuous	Geothermal water
10.11. – 29.11.2010	Batch	Mineral medium
29.11. – 10.12.2010	Continuous	Geothermal water

4.1. Nitrification

The nitrification Lentikats Biocatalyst was re-activated using mineral medium of optimal composition to achieve the activity of 70 mg N/kg LB/hr. This value corresponds to the average values achieved under similar conditions (temperature 10-13°C). Upon the replacement of the mineral medium by pre-treated geothermal water, the Biocatalyst's activity decreased to an average of **30-50 mg N/kg LB/hr**, which already indicated a possible inhibition of the Biocatalyst. The temperature during these adaptation batches gradually increased to 17°C. At this temperature and with the initial concentration of about 25 mg N-NH_4/L, the Biocatalyst normally achieves activities of about 250 mg N/kg BL/hr in normal municipal wastewater free of potentially inhibiting substances.

Time course of the Lentikats Biocatalyst's activity and associated effluent N-NH_4 concentrations in the nitrification reactor is presented in graph 1. As can be seen, during the first continuous operation phase the Biocatalyst's activity decreased from the initial re-activation level of 32 mg N/kg LB/hr and stabilised at the level of 10 mg N/kg LB/hr. The average temperature during this phase was 22°C and the average N-NH_4 concentration in the pre-treated geothermal water 26.7 mg/L. As discussed earlier, the caustic overdose on October 26 caused a severe damage to the Biocatalyst, which shows in the fast deteriorating activity of the Biocatalyst during the second continuous operation. The Biocatalyst was then re-cultivated. However, due to the limited time available for this project, the re-cultivation was not complete and the Biocatalyst regained only 50% of its initial activity, i.e. 15 mg N/kg LB/hr. Once switched to the continuous operation mode with real geothermal water, the Biocatalyst's activity yet again dropped to a zero level. The most likely cause of this failure is a combination of still insufficient bacterial population inside the Biocatalyst with an inhibiting effect of the geothermal water.



The amount of results obtained during the test is insufficient and does not allow drawing of any firm conclusions. Taking into account the data obtained during the re-activation and first continuous phase, the results suggest a strongly inhibiting effect of the geothermal stream on the encapsulated nitrification biomass. Minimum amount of nitrites formed during the operation indicates that the inhibition affected mostly the first phase of nitrification, i.e. nitritation. Out of the two groups of organisms involved in the nitrification, the nitritation *Nitrosomonas spp.* is often reported to be more susceptible to inhibition by heavy metals

(Henze *et al*. 2002). However, the most common inhibiting compounds and elements reported for this species in literature (Table 1) were present in the geothermal stream at concentrations below their inhibiting level. Moreover, the previous inhibition potential test reported by Vega *et al.* (2007) did not show any inhibition effect of the same geothermal water on the nitrification activity of activated sludge samples. Those tests were however carried out as a one-off measurement, which disregard a potential cumulative inhibition, a possible cause of the Biocatalyst's low activity during the here presented trial. Due to the limited time available for the trial, the cause of inhibition could not be identified. A potential adaptation of the microorganisms to this inhibitory component, a phenomenon previously reported for nitrification Lentikats Biocatalyst (Boušková *et al.* 2009), could not be evaluated for the same reason.

4.2. Denitrification

Denitrification Lentikats Biocatalyst achieved initially an average activityy of 53 mg N/kg LB/hr during the reactivation with standard mineral medium. Again, this value corresponds to the average values obtained under similar conditions elsewhere. The Biocatalyst retained this activity during the batch tests performed with real geothermal water indicating a successful adaptation of the bacteria to the real water.

Once switched to the continuous operation mode, the inhibition caused to the nitrification Biocatalyst resulted in a very low production of nitrates for denitrification. The denitrification reactor achieved a steady zero effluent concentration of N-NO_x, which disallowed any accurate evaluation of the Biocatalyst's activity.

The NaOH overdose did not have any deteriorating effect on the denitrification bacteria as the Biocatalyst achieved an activity of 108 mg N/kg LB/hr during the November re-cultivation. Once switched to the continuous operation on November 29, the denitrification Biocatalyst was for the first few days receiving an inflow with high concentration of nitrates (accumulated in the nitrification reactor during the re-cultivation batch operation). Despite these high concentrations of nitrate, the denitrification Biocatalyst kept on achieving a zero level or nitrates in the effluent, thus indicating an activity of more than **270 mg N/kg LB/hr**. Taking into consideration the average temperature of 26 °C and inlet concentrations of 20-70 mg N-NO_3/L, such activity corresponds to the values observed under similar conditions in common municipal wastewater and yet again proving no detrimental effect of the real water on denitrification bacteria.

The low concentrations of nitrates coming into the denitrification tank from the nitrification reactor made it impossible to accurately determine and optimise the dose of external carbon source (ethanol) required for successful denitrification. The data obtained during the re-activation batch tests suggested an optimal dose of less than 2 mg COD/mg N-NO_3, while the data obtained during the last continuous phase of the trial gave a value of 17 mg COD/mg N-NO_3. While the former value is below the stoichiometric value for denitrification of 4.2 mg COD/mg N, the latter value is spoilt by a high DO concentration of the treated water. The denitrification bacterium firstly utilises the dissolved oxygen as an electron donor while reducing the organic carbon before it "attacks" the nitrates. Therefore, the experimentally determined value of 17 mg COD/mg N-NO_3 is much higher than expected. This issue can be overcome by effective regulation of the aeration inside the nitrification tank.

5. CONCLUSIONS

The efficiency of Lentikats Biotechnology for the removal of ammonia nitrogen from geothermal water was tested in a pilot-scale trial. The test results indicated an inhibiting effect of the geothermal water on the nitrification bacteria, yet the real source of inhibition was not possible to identify due to the limited time available for the project. Also, potential adaptation of the nitrification Biocatalyst to such inhibition can be expected, but further testing would have to be carried out to confirm this assumption. The denitrification

Biocatalyst achieved activity similar to those obtained under similar conditions in conventional wastewaters, i.e. no inhibiting effect was observed in this case.

The test results obtained so far suggest that the amount of nitrification Lentikats Biocatalyst necessary for full-scale oxidation of ammonia from the geothermal stream would be 9 times higher than the amount indicated in the proposal presented to RDC in May 2009. Such process would probably be economically unviable. However, the

Denitrification Biocatalyst is likely to achieve its suggested activity under stable loading and operation and the cost associated with this part of the treatment process would therefore remain as stated in the preliminary proposal.

Further testing (at a smaller scale) of the adaptability of nitrification Lentikats Biocatalyst to the geothermal water is proposed in order to verify the up to date results and assumptions.

6. REFERENCES

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7. APPENDIX

Appendix 1. Test unit scheme