Introduction of Specialised Micro-organisms for the Enhancement of Bioremediation at Shing Mun River

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Abstract

Many lakes, rivers and other water bodies have been polluted with organic wastes from various sources. In densely populated areas such as Asia, effluents cause a negative impact not only on sediment, but also on the water column. Problems of eutrophication and particularly odour arise.

Since 2001, the Hong Kong Government has launched a five-year programme to bioremediate the sediment of Shing Mun River. Nitrates were injected into the sediment to oxidise sulphides and to initiate the aerobic degradation of organics. This process had relied on the degradative activity of the indigenous micro-organisms. Odour problem has been reduced, but the rates of Total Organic Carbon (TOC) breakdown have yet to be assessed.

This Paper describes a new method of supplementing the bioremediation. Specially selected natural micro-organisms were introduced to the sediment (in-situ) at two locations. A bench scale water trial was also carried out in five tanks which represented, at scale, the river conditions, of both the water column and the sediment. A range of qualitative and quantitative analysis was performed prior to and after treatment.

The results show that the added micro-organisms were active in both water and sediment. This was seen from the more negative redox and the decreased nitrate levels in the in-situ sediment trial and the initial drop of DO, the changes in COD levels and various nitrogen forms at different treatment times in the bench-scale water trial. Improved sediment settling characteristics, which will decrease nutrients cycling from sediment to water, was also observed in the bench-scale water trial.

Improvements in sediment qualities, though noticeable, were not quantified as the duration of trial was too short. For more efficient removal of TOC, it is recommended that microorganisms should be injected along with the nitrate solution. This would ensure a more thorough mixing among the added micro-organisms, the nitrate and the pollutants to be treated.

Keywords
Bioremediation, natural micro-organisms, nitrate, sediment

1. Introduction

In 2001, Civil Engineering Department (CED) introduced an in-situ remediation technology using nitrate injection to treat the sediment at Shing Mun River (SMR). (See report by TSUI et al, 2001.) Part of the injected nitrate was quickly consumed during the sulphide oxidation process to mitigate the odour problem. The remaining nitrate would initiate the aerobic degradation of organics by indigenous micro-organisms in SMR for the long term control of odour. Since the sediments were anaerobic at initial stage, it would take time for the indigenous micro-organisms to initiate the biodegradation process.

To speed up the process, Environmental International Limited (EIL), in conjunction with CED, applied various strains of cultured natural micro-organisms to the sediment in 2003. Two trials were executed, one for in-situ sediment and the other for bench-scale sediment/water.

2. Summary of Trials

This section describes only the key elements of the trials. For details of the trials, please refer to the proposal entitled "Study of Enhanced Bioremediation of Shing Mun River February, 2004" by CED/EIL, which is available upon request.

Two microbial products were utilised in the trials:

- PC I - For the remediation of water
- PCII - For the remediation of sediment

2.1 Sediment Trial (in-situ)

2.1.1 Site Description and Treatment Programme

Table 1 shows the details of the sites and the treatments applied.

Site	Description	Dosage
Siu Lek Yuen (SLY)	Test area of 50m x 30m. Area was never treated with nitrate.	50g /m^2 PC II at day 1 (total: 75kg) 25g /m^2 PC II at day 14 (total: 37.5kg) 25g /m^2 PC II at day 28 (total: 37.5kg)

Man Lai Court (MLC)	Test area of 50m x 70m. Area was treated on one occasion with 1000mg/m^3 of $Ca(NO_3)_2.4H_20$ about 15 months ago.	50g /m^2 PC II at day 1 (total: 175kg) 25g /m^2 PC II at day 14 (total: 87.5kg) 25g /m^2 PC II at day 28 (total: 87.5kg)

Table 1 - Site Description and Treatment Programme

2.1.2 Sampling Points

Table 2 shows the sampling details. For each site, there were eight samples within the treated boundary. They were composited into four samples. Control was taken as one composite made up from four sampling points.

Name	Treated points within boundary	Composite Samples (Treated)	Composite Sample (Control)
Siu Lek Yuen (SLY)	1-8	(1,7), (2,5), (3,8), (4,6)	(9-12)
Man Lai Court (MLC)	1-8	(1,5), (2,7), (3,6), (4,8)	(9-12)

Table 2 - Sampling Details

2.1.3 Testing Frequency and Parameters

A series of core samples were taken the day before the first dosage, and tested. The sampling and testing were repeated approximately two weeks after the final dosage. Tested parameters included Redox, Nitrite, Nitrate, TOC and *E. Coli.*.

2.2 Water Trial (bench-scale)

2.2.1 Experimental setup

Whilst the in-situ sediment trial aimed to enhance biodegradation within the river-bed, a bench-scale water trial was also carried out, aiming to observe and record such enhancement, if any. As the bench-scale trial was carried out under control situations in tanks, such external factors as new pollution loads (to the river-bed) and tidal influences could be reduced or eliminated. The bench-scale trial also aimed to ascertain if improvements in the overlying water column could be achieved. Details of the experimental set-up are as follows:

- 5 x 1m^3 tanks were employed.
- 3 tanks contained sediment and 2 did not.
- The ratio of sediment to water in Tanks T1, T2, and T3 was 0.272:1 (simulating the ratio at Siu Lek Yuen (SLY) based on average data at high and low tides).
- Water cycled once every 24 hrs in T3 according to the ratio of 0.18 (depth of water replacement): 0.71 (total water depth) which simulated the tidal influence at SLY.
- Dosage times were 14 days apart for T2 and T4, with an 11 day interval for T3 due to need of water cycling.

Summary of the experimental setup is shown in Table 3.

Name	Description	Dosage
T1 (Control)	Contains sediment and water	X
T2	Contains sediment and water	Initial dosage 50g PC II 25g PC 1 2 further doses 25g PC II 10g PC I
T3	Contains sediment and water with water cycling at intervals to simulate tidal effect. A measured quantity of water is removed and replaced with water from SMR once every 24hrs.	Initial dosage 50g PC II 25g PC 1 2 further doses 25g PC II 10g PC I
T4	Water only with no sediment	Initial dosage 25g PC 1 2 further doses 10g PC I
T5 (Control)	Water with no sediment	X

Table 3 - Summary of Experimental Setup

2.2.2 Testing Frequency and Parameters

One water sample was taken from each tank on Day 0, 14, 28 and 42. Tested parameters included pH, Dissolved Oxygen (DO), Temperature, Turbidity, Total Suspended Solid (TSS), *E. Coli.*, Total Bacteria, Ammonia, Nitrite, Nitrate, Total Phosphorus, Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and Chlorophyll *a*.

2.2.3 Post-agitation Settling Test

To assess if there were improvements in the settling characteristics of the sediment treated, additional settling tests were carried out at the end of the trial on Day 42. A water sample for Total Suspended Solid (TSS) analysis was taken from each of the tanks T1, T2 and T3 prior to agitation. The surfaces of the sediments in the tanks were then stirred and allowed to settle. About 30 minutes later, another water sample was taken from each of the tanks and analyzed for its TSS. The same procedure was repeated about another hour later. In reality, samplings were taken at 15:20 pm, 16:00 pm and 17:15 pm.

3.0 Results and Findings of Sediment Trial

3.1 Redox

Figure 1 and 2 show the pre- and post-treatment redox at SLY and MLC respectively. On average, redox was more negative at most of the sampling points after the addition of PCII at both sites.

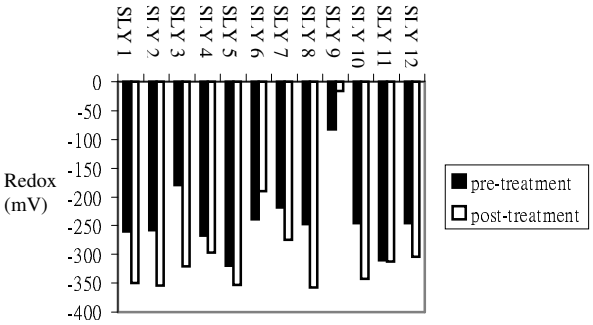

Figure 1 - The Pre- and Post-treatment Redox at Different Sampling Points of SLY

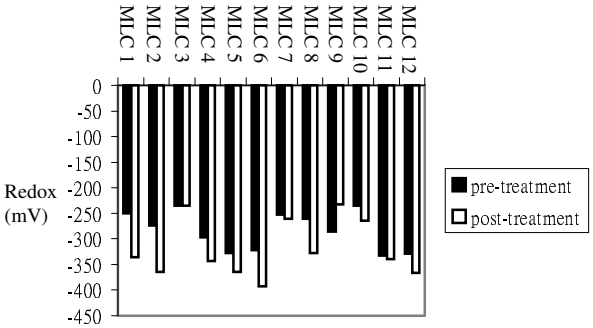

Figure 2 - The Pre- and Post-treatment Redox at Different Sampling Points of MLC

From Figures 1 and 2, we formulated Table 4, the specific reduction rate of redox. We note from the table that the specific reduction rate of redox were 20.35% and 17.27% for SLY and MLC, respectively.

Site	Average Reduction in Redox (mV) [1]		Specific Reduction Rate of Redox (%) [2]		Specific Reduction Rate of Redox (%) off-setting Control [3,4]
	Treated	Control	Treated	Control	
SLY	-63.34	-22.65	25.47	10.23	20.35
MLC	-50.56	-5.26	18.22	1.90	17.27

Table 4 – Specific Reduction Rate of Redox

[1] Average Reduction in Redox (mV) = Sum of Reduction in Redox of All Treated or Control Samples / Number of SamplesSpecific

[2] Specific Reduction Rate of Redox (%) = Average Reduction in Redox of Treated or Control Samples / Average Pre-treatment Redox of Treated or Control Samples

[3] Specific Reduction Rate of Redox (%) off-setting Control = Specific Reduction Rate of Redox (%) of Treated Sample – (Specific Reduction Rate of Redox (%) of Control Sample x 50%)

[4] This 50% was assumed due to that the reduction in control would be half by new pollution load and half by possible drifting of PCII over the control area

The reduction in redox demonstrated that there had been enhancement of biodegradation process. Within this process, oxygen (from various chemical sources) was consumed thereby reducing the redox. At the MLC site the residual nitrate provided the chemical oxygen source and was used up by the added micro-organisms. This resulted in the redox becoming more negative during the trial. At the SLY site, which did not have nitrate injected, the added micro-organisms used other available chemical oxygen sources e.g. phosphates, sulphates, carbonates, etc.

3.2 Nitrate

Figures 3 and 4 show the pre- and post-treatment nitrate values at SLY and MLC respectively. It is clear that the addition of PCII had greatly reduced the level of nitrate in the sediment at both sites.

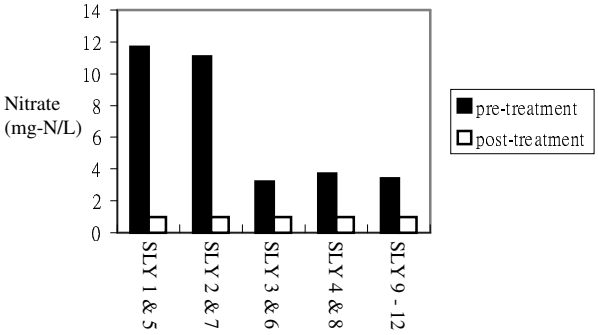

Figure 3 - The Pre- and Post-treatment Nitrate at Different Sampling Points of SLY

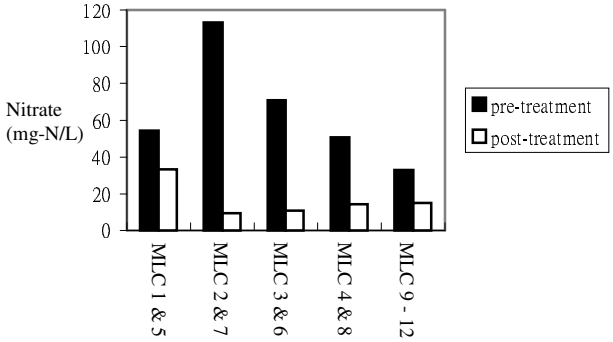

Figure 4 - The Pre- and Post-treatment Nitrate at Different Sampling Points of MLC

From Figures 3 and 4, we formulated Table 4, the specific reduction rate of nitrate. We note from the table that the specific reduction rate of nitrate were 49.60% and 46.98% for SLY and MLC, respectively.

Site	Average Reduction in Nitrate (mg-N/L) [1]		Specific Reduction Rate of Nitrate (%) [2]		Specific Reduction Rate of Nitrate (%) off-setting Control [3,4]
	Treated	Control	Treated	Control	
SLY	5.62	2.40	84.89	70.59	49.60
MLC	47.76	18.00	74.25	54.55	46.98

Table 5 – Specific Reduction Rate of Nitrate

[1] Average Reduction in Nitrate (mg-N/L) = Sum of Reduction in Nitrate of All Treated or Control Samples / Number of Samples

[2] Specific Reduction Rate of Nitrate (%) = Average Reduction in Nitrate of Treated or Control Samples / Average Pre-treatment Nitrate of Treated or Control Samples

[3] Specific Reduction Rate of Nitrate (%) Off-setting Control = Specific Reduction Rate of Nitrate (%) of Treated Sample – (Specific Reduction Rate of Nitrate (%) of Control Sample x 50%)

[4] This 50% was assumed due to that the reduction in control would be half by new pollution load and half by possible drifting of PCII over the control area

At the MLC site the pre-treatment nitrate level was higher than that at the SLY site due to the previous injection of Calcium Nitrate at MLC. The reduction in nitrate at both sites clearly showed that the biodegradation process was enhanced. Nearly 50% of nitrate was consumed within the 2 months trial period. As there should not be any significant nitrate consumption by indigenous micro-organisms in SMR within 2 months time due to TOC breakdown, such nitrate depletion was believed to be caused by the added micro-organism, which used up nitrate effectively for further biodegradation.

3.3 TOC

Figures 5 and 6 show the pre- and post-treatment TOC at SLY and MLC respectively. Against our expectation of TOC removal, slight increase of TOC was observed at most of the sampling points after the addition of PCII at both sites.

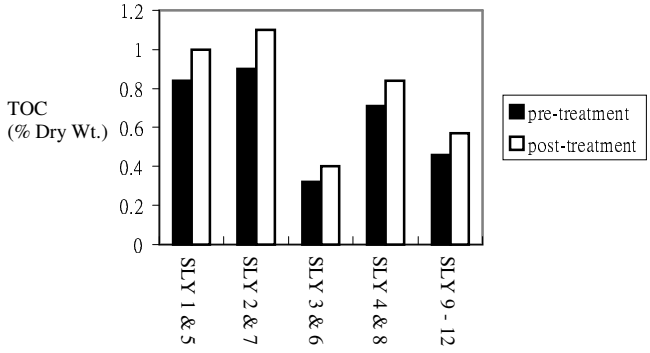

Figure 5 - The Pre- and Post-treatment TOC at Different Sampling Points of SLY

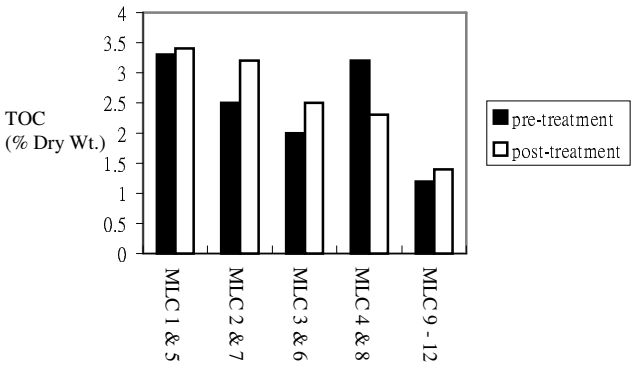

Figure 6 - The Pre- and Post-treatment TOC at Different Sampling Points of MLC

No removal of TOC might be due to the following reasons. Firstly, due to the short duration of trial, the removal of TOC cannot be quantified since the organics remained in the sediments of SMR are long chain carbon and require longer time to be degraded. Furthermore, the removal activities of the added micro-organisms, which were delivered by natural calcified carriers to the river-bed, were initiated only at the water sediment interface. A longer treatment time is required for the micro-organisms to work downwards for further degradation of those organics deep down in the sediment. The increase of TOC in the control can be concluded that there was an increase of sedimentation and organics over the sampling points at both sites during the trial.

4. Results and Findings of Water Trial

4.1 Dissolved Oxygen

Table 6 shows the DO levels prior to and after PCI & II addition on Day 14.

Name	Sampling Date (prior to addition)	DO (mg/L)	Sampling Date (after addition)	DO (mg/L)
T1	Day 0	9.34	Day 14	7.08
T2	Day 0	10.79	Day 14	0.11
T3	Day 0	10.34	Day 14	0.23
T4	Day 0	9.81	Day 14	0.08
T5	Day 0	9.99	Day 14	5.33

Table 6 - DO Levels

At initial stage (i.e. Day 14), DO was greatly reduced in Tanks T2, T3 and T4 after addition of PCI & PCII. The treated tanks exhibited much lower levels of DO from 0.08 to 0.23 mg/L. This indicated that microbial

activities were vigorous. In contrast, the controls T1 & T5 exhibited no such reduction. Aeration was then introduced to the experimental tanks on Day 15. The DO levels of all experimental tanks after aeration were kept between 5 and 8 mg/L.

It should be pointed out that in reality when PCI & II are added to SMR, there should be no need to aerate the water, as turbulence in flowing water will play a role in the transport of oxygen into the water column.

4.2 COD

Figure 7 shows the COD levels prior to and after PCI & II addition during the trial.

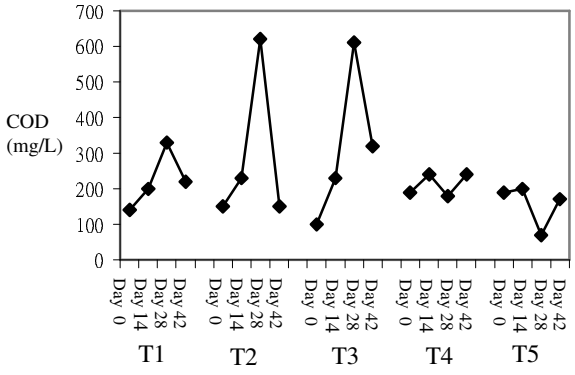

Figure 7 - COD Levels

Tanks T1, T2 and T3 which contained sediment showed an increase of COD levels from Day 0 to 28. The rises were particularly high for the treated tanks T2 and T3, up to about 600 mg/L. Towards Day 42, the COD levels of T2 and T3 dropped to about 100 mg/L and 300 mg/L respectively. Tanks T4 and T5 which did not contain sediment did not show the same trend.

The increase of COD levels in T2 and T3 was due to the microbial action from the added micro-organisms. They started to break down the accumulated organics accumulated in sediments and released some colloidal materials into the water. Contrast to T2 and T3, the control T1 showed no obvious increase. The subsequent fall in COD on Day 42 was due to the settling and stabilization of the released colloidal materials and the broken down of most of organics in water and sediment. We noted at tanks without sediment, there had been no obvious change of COD levels. This was due to the fact that the above releasing of colloidal materials from sediment did not happen.

4.3 Nitrogen Forms

Figure 8 shows the different nitrogen forms prior to and after PCI & II addition during the trial.

At initial stage (i.e. from Day 0 to Day 28), ammonia was observed to rise with nitrate dropping in all the treated tanks T2, T3 and T4. Towards Day 28, the reverse occurred. The highest ammonia recorded was 5 mg/L at T2 on Day 28. Depletion of nitrate was found to be obvious at the treated tanks, from 1.5 -2.0 mg/L to near zero at Day 14 or 28. Nitrite level behaved somewhat similar to nitrate.

The initial rise of ammonia in all treated tanks indicated that the added micro-organisms were degrading proteins and other nitrogen containing organics. As these organics were degraded, the conditions for natural nitrification by indigenous nitrifying bacteria were improving, resulting in the conversion of ammonia to nitrites and nitrates. This conversion is sensitive to organics and the growth rate of indigenous nitrifying bacteria are generally higher in low organics environment. In all, the added micro-

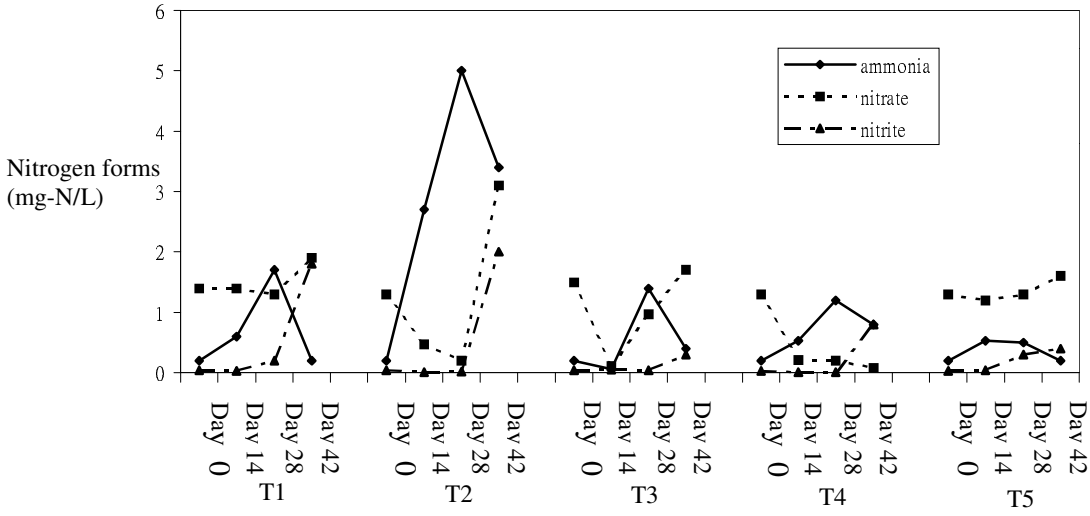

Figure 8 – Ammonia, Nitrite and Nitrate Levels

organisms degraded not only the organics, but also enhanced the natural nitrification processes and worked in total harmony with the indigenous bacteria in the river ecosystem of SMR.

4.4 Post-agitation Settling Test

Figure 9 shows the TSS of T1, T2 and T3 that contained sediment, prior to and after agitation.

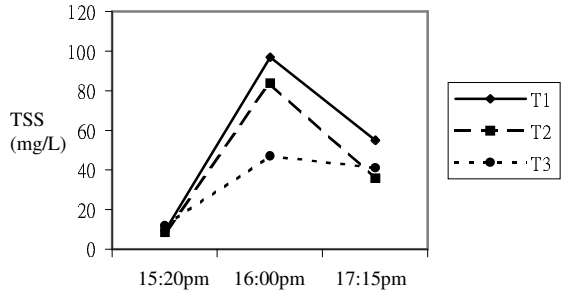

Figure 9 - TSS Levels of Tanks T1, T2 and T3

All the Tanks T1, T2 and T3 showed an increase of TSS levels at 16:00 pm, about 30 minutes after agitation. The increase was particularly high at the untreated tank T1, up to about 100 mg/L. At 17:15 pm, the TSS levels of all the tanks dropped. The TSS levels of T2 and T3 at 17:5 pm were lower than that of the untreated tank T1.

We noted that the settling characteristics of the sediments in T2 & T3 had improved and their settling time was reduced. This is important because disturbance of the sediment in the river due to boat activities and tidal influences will recycle nutrients from the sediment into the water body, which has been known to have a direct effect on the proliferation of algae and eutrophication. Therefore, the improved settling characteristics of the treated sediment can shorten the retention time of nutrients in the water body, thereby decreasing nutrient available to algae.

5. Conclusions

We conclude that:

i.) the added micro-organisms from PCI & PCII were active in both water and sediment due to the more negative redox and the decreased nitrate levels in the in-situ sediment trial and the initial drop of DO, the changes in COD levels and various nitrogen forms at different treatment times in the bench-scale water trial;

ii.) the conditions for natural nitrification of indigenous nitrifying bacteria were improved by the added micro-organisms; and

iii.) the settling characteristics of sediments were improved, which will decrease nutrients cycling from sediment to water.

6. Recommendations

Notwithstanding the foregoing improvements, we did not record obvious improvements in the TOC removal rate. Whilst the short duration of the trials could be the main reason, we believe there are rooms for improvement for example by improving methods to apply micro-organisms into the sediment. For more efficient removal of TOC in SMR, we recommend to make use of biovessel to deliver the natural micro-organisms to the desired treatment zone in a control manner.

7. Reference

TSUI, T.S., LEUNG K.M, CHUI, H.K., WONG, H.W., 2002, Bioremediation of Sediment of Shing Mun River, *Proceedings of International Conference on Innovation and Sustainable Development of Civil Engineering in the 21st Century*, Beijing, China