MICROBIAL DYNAMICS OF THE MAIN BACTERIAL GROUPS IN AN ANAEROBIC POND TREATING LANDFILL LEACHATE

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INTRODUCTION

Sanitary Landfill

Widely used method for solid waste disposal

Brazilian Sanitary Landfill

Operational Implementation Management

Not defined yet!

Landfill Leachate

High concentration of organic matter, nutrients, pathogens ...

If treated incorrectly

Serious pollution problems to surface and groundwater
INTRODUCTION

Diversity of organisms (bacteria and algae) regulated treatment process.

Several physical, chemical and biological processes.

Stabilization Ponds

Recycling of organic matter and nutrient by microorganisms.

Diversity of organisms (bacteria and algae) regulated treatment process.

Several physical, chemical and biological processes.

TREATMENT

Several physical, chemical and biological processes.

Stabilization Ponds

Recycling of organic matter and nutrient by microorganisms.

Diversity of organisms (bacteria and algae) regulated treatment process.

Evaluate their interaction with the environment and microorganisms.

Present work → Studied the anaerobic pond of a serial pilot system (anaerobic, facultative and maturation ponds) aiming to characterize the microbial community and corelated them to physical-chemical parameters.

Fisiology

Show the role of each microorganism in the different treatment steps.
MATERIALS AND METHODS

→ Origin and characteristics of leachate

• Leachate → Sanitary landfill – Proactiva – Biguaçu/SC – Km 177 (BR 101).

• In operation since 1991
• Receives urban solid waste from 14 cities around Florianópolis = 14 thousand t/month
• Leachate drained and conducted → equalization tank

Fiberglass tank (5,000 L)
MATERIALS AND METHODS

→ Localization

- Experimental unit → LABEFLU/LARESO – UFSC, Florianópolis.

→ Pilot System

- Pond 1 (AP) → anaerobic
- Pond 2 (FP) → facultative
- Pond 3 (MP) → maturation

Biological treatment (serial pilot system)
MATERIALS AND METHODS

→ Physical characteristics of anaerobic pond (AP)

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (m)</td>
<td>2.0</td>
</tr>
<tr>
<td>Diameter (m)</td>
<td>1.85</td>
</tr>
<tr>
<td>Volume (m³)</td>
<td>5.0</td>
</tr>
<tr>
<td>Hydraulic Detention Time (d)</td>
<td>25</td>
</tr>
</tbody>
</table>

- The system was in operation since August/2007 to May/2008. The influent rate was 200 L/day.

→ Samples and Monitoring

- The samples were collected every 15 days, at 2:00 pm.

Measured in situ:
- PH
- ORP (mV)
- Conductivity (mS/cm)
- Temperature (⁰C)

Measured in laboratory:
- Dissolved Oxygen (mg/L)
- Ammonium (mg/L)
- Chemical Oxygen Demand (COD)
- Biochemical Oxygen Demand (BOD₅)
- Suspended Solids (SST)

Probe YSI 6600
APHA, 2005
**MATERIALS AND METHODS**

→ *Microbiological Analysis*

**FISH - Fluorescent in situ Hybridization**

The probes were used to measure the *in situ* growth rates based on Amann (1995).

Involves oligonucleotide probes application to permeabilized microbial cells and specifically hybridized the cells with their complementary target sequence.

<table>
<thead>
<tr>
<th>Probe name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB mix (I+II+III)¹</td>
<td>5' GCTGCCTCCCCCCGTAAGAA</td>
</tr>
<tr>
<td>NEU¹</td>
<td>5' CCCCTCTGCTGCAACGTG</td>
</tr>
<tr>
<td>AMX820¹</td>
<td>5' AAAACCCCTCTAGTCCG</td>
</tr>
<tr>
<td>Eury 499¹</td>
<td>5' CCGTCTTGGCCCGGCGCG</td>
</tr>
<tr>
<td>DSV 407¹</td>
<td>5' CCAGAGGCTCTTGCG</td>
</tr>
<tr>
<td>ARC 915</td>
<td>5' GTCGGCCGCGCGGCGCG</td>
</tr>
<tr>
<td>NSO 190</td>
<td>5' CGATCCCTGCTTGCGCG</td>
</tr>
</tbody>
</table>
**MATERIALS AND METHODS**

→ *Microbiological Analysis*

**DNA Extraction**

Performed by Leão et al. (1999)

- 400µL TE/Triton 1%
- 3 alternate cycles of boiling and freezing (10 minutes each)

**Polymerase Chain Reaction (PCR)**

- Primer (677–1112 / 16S rDNA)
- 436For (5’ – GAGCGGTGAAATGAG – 3’)
- 436Rev (5’–GACGGGCGGTGTATAAG – 3’)

The reaction was analysed by 1% agarose gel electrophoresis stained with ethidium bromide (0.5µg/mL), visualized under Uvlight (Sambrook et al., 2001).

**DNA sequencing and phylogenetic analysis**

- The amplified DNA was purified with DNA purification Kit (GE Healthcare, UK) following manufacturer’s protocol;
- Sequencing was performed in a 3130 XL automatic sequencer (Applied Biosystems);
- The quality of sequences was checked and compared to sequences deposited in GenBank/NCBI. Comparative analysis by specific software.

**Amplification (Mastercycler Eppendorf)**

- 3 min - 95°C
- 45 s - 55°C
- 1 min - 60°C
- 1 min - 72°C
- 7 min - 72°C
- 30 cycles

- The reaction was analysed by 1% agarose gel electrophoresis stained with ethidium bromide (0.5µg/mL), visualized under Uvlight (Sambrook et al., 2001).
### RESULTS AND DISCUSSION

→ **Physical-chemical parameters**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAW</td>
</tr>
<tr>
<td>Suspended Solids (mg/L)</td>
<td>440 ± 178</td>
</tr>
<tr>
<td>T (°C)</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>8.8 ± 0.2</td>
</tr>
<tr>
<td>Conductivity (mS/cm)</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>ORP (mV)</td>
<td>-313 ± 42</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>3200 ± 739</td>
</tr>
<tr>
<td>BOD$_5$ (mg/L)</td>
<td>1268 ± 607</td>
</tr>
<tr>
<td>N-NH$_4$ (mg/L)</td>
<td>1145 ± 234</td>
</tr>
</tbody>
</table>

Removal of: 21% for COD and Ammonium; and 35% for BOD
RESULTS AND DISCUSSION

→ FISH – Hibridização Fluorescente in situ

Probes
- EUBmix (Bacterias)
- NEU (Nitrosomonas sp.)
- NIT (Nitrobacter sp.)
- NSO (beta proteo oxid.)
- ARC (Archae)
- Eury (Methanomicrobiales)
- DSV (Desuifovibionaceae)
- AMX (Anammox)

Rare occurrence (↓OD; ↑pH)
- Until 35% (January/2008)

Also found
- sulfate reduction and producing sulfide

Absent
- 80% (initial months until Aug/Dec 2007)

30% (↓Metabolic activity)

Cells hybridized – analysis of Eubacteria:
- DAPI (100%)
- EUBmix (≅70%)

Cells hybridized – analysis of SRB:
- DAPI (100%)
- SRB (≅30%)
RESULTS AND DISCUSSION

Sequencing and phylogenetic analysis

Sequences with more than 98% similarity
The landfill leachate treated in the AP presents favorable physical-chemical characteristics and can be transferred to a second biological treatment stage (aerobic ponds);

The FISH technique identified a variety of bacterial species developed spontaneously and involved in the anaerobic degradation process of organic matter, including hydrolytic, fermentative acidogenic, acetogenic, sulphate-reducing and methanogenic bacteria. Groups Anammox weren’t detected in the present study;

By phylogenetic analyses, Pseudomonas genera were identified;

Studies of microbial dynamics associated with physical-chemical oscillations, can contribute to validate the control of effluents and improve future system management.
THANK YOU !!!!!