The effect of environmental conditions on faecal coliform decay in post-treatment of UASB reactor effluent

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Abstract: The removal of faecal coliforms (FC) in waste stabilization ponds is partly caused by natural decay processes. This work distinguishes between light mediated and light independent processes, since only the upper layer of a stabilization pond receives solar radiation. Light attenuation by algae matter or other particles causes darkness in the rest of the pond. The purpose of this work was to investigate the decay processes in stabilization ponds in order to yield improved design of pond systems.

Experiments were carried out with buffered effluents from an Upflow Anaerobic Sludge Blanket (UASB) reactor, treating domestic wastewater. The FC decay rate was determined and compared for light and dark conditions, in aerated bottles and beakers, respectively. The effective environmental factors were also investigated and mathematical expressions were derived for their effect on the FC decay rate. Special attention was given to the effect of light attenuation by algae matter.

It was found that light independent FC decay in aerated UASB effluent is caused by a shortage in carbon sources, since glucose addition prevented decay for over ten days. The nutrient content of UASB effluent was satisfactory for a long-term survival. The FC decay in the dark parts of stabilization ponds is therefore under conditions of carbon and nutrient sufficiency expected to be negligible. Under conditions of carbon shortage, the light independent FC decay was found to be temperature dependent, but not pH dependent (in the range 7.2–9.1).

The FC decay in beakers exposed to solar radiation was much faster then under dark conditions. The light mediated decay was affected by the pH, DO concentration and the solar radiation intensity, but not by the temperature. The addition of autoclaved algae matter strongly reduced the FC decay in the beakers, due to light attenuation. It is therefore expected that the light attenuation by algae matter in stabilization ponds also reduces the FC decay. This could partly offset the stimulating effects of algae photosynthesis (i.e. increased pH and DO concentrations) on FC decay. It seems therefore that there is an optimum algae concentration for maximum FC decay. This can lead to a new design approach, based on regulation of algae growth in stabilization pond systems.

Keywords: Faecal coliforms; pathogen decay; reuse; stabilization ponds; UASB reactor

Introduction
Wastewater stabilization ponds are widely applied treatment systems that are known for effective removal of pathogenic bacteria (Reed et al., 1995). Removal efficiencies are usually measured in terms of a group of non-pathogenic indicator bacteria, called faecal coliforms (FC). The removal of FC in stabilization ponds is due to very complex interactions of physical, chemical and biological processes (Polprasert et al., 1983; Curtis et al., 1992a, b). Reed et al., (1995) mention natural decay, predation, adsorption to solids and sedimentation as possible removal processes. The relative importance of those processes has hardly been reported in literature. Adsorption to suspended solids and subsequent sedimentation in stabilization ponds is probably of minor importance (Sarikaya et al., 1987b), especially after pre-treatment in an anaerobic pond or anaerobic reactor. In this work it is also assumed that the predation is a negligible mechanism, due to the limited possibilities for protozoa adsorption, as compared to activated sludge systems. Predation of bacteria by protozoa in
activated sludge systems was reported to be an important process for FC removal (Frijns and Lexmond, 1991). The most significant mechanisms for FC decay are therefore probably natural decay processes, including: (1) DNA damage caused by the UV component of solar radiation (Curtis et al., 1992a, b; Moeller and Calkins, 1980); (2) photo-oxidation (Curtis et al., 1992a, b); (3) bactericidal effects of algae growth (Parhad and Rao, 1974) and (4) starvation due to lack of nutrients or carbon source.

The natural decay processes were found to be affected by environmental factors, such as pH, temperature, dissolved oxygen (DO) concentration, solar radiation, algae, substrates and nutrient concentrations (Frijns and Lexmond, 1991). In the literature often no distinction is made between the effects of environmental conditions on light mediated and on light independent decay processes. This distinction is however important to understand the FC decay in stabilization ponds, because each pond is divided in an upper layer that receives solar radiation, and a lower layer that remains dark. The thickness of the radiated zone is determined by the light attenuation by algae and other particles. It is generally 10-30 cm deep (Pearson et al., 1987).

Previous studies on environmental factors affecting FC decay considered the following factors to be important and selected them as key parameters for model development: temperature (Marais, 1974), solar radiation averaged over the pond depth (Sarikaya et al., 1987a, b; Mayo, 1989), temperature and salinity (Mancini, 1978) and UV-radiation (Moeller and Calkins, 1980). Two models have suggested that FC decay is affected by many environmental factors such as temperature, algae concentration, light intensity and organic loading (Polprasert, 1983) or by pH, temperature, organic loading, turbidity and algae concentration (Qin, 1991).

The purpose of the present work was to investigate the effects of environmental factors on FC decay under dark and light (solar radiation) conditions. The experiments were conducted with effluents obtained from a UASB reactor, treating domestic sewage. Therefore the results presented are applicable for the post-treatment of UASB reactor effluent. The relative importance of the environmental factors has been estimated by establishing mathematical relationships between each environmental factor and the decay coefficient (as will be defined later). Recommendations are made for a new design approach for waste stabilization pond systems.

**Materials and methods**

Experiments were conducted with effluents from a UASB reactor that treated domestic wastewater. The BOD$_5$ of the effluents was in the range 10–50 mg/l and the FC counts $10^5$–$10^6$ per 100 ml.

**Dark environment experiments**

The decay of FC in a dark environment under various environmental conditions (Table 1) was studied in 1 litre bottles, covered with black plastic. The bottles were placed in a temperature controlled water bath. The bottles were filled with 900 ml UASB effluent and 100 ml 1M phosphate buffer. The bottles were continuously aerated in order to maintain oxygen saturation of the effluent, and for mixing. Samples were taken during the experiments, stored at 4°C and analyzed within 3 hours for FC, according to Standard Methods filtration method (APHA, 1995). Evaporation losses were compensated with demineralized water. The pH was measured regularly in all experiments; in experiment D.1 BOD$_5$ was measured several times.
Outdoor experiments to study the effect of direct solar radiation under various environmental conditions (Table 1) were carried out in 1 litre glass beakers placed in a temperature controlled water bath. Sides and bottom of the beakers were covered with black plastic, but the surface area was exposed allowing direct solar radiation from above. The beakers were filled with the same effluents as used for the experiments in the dark environment. The mixture was buffered with either phosphate- or TRIS-buffer [tris-(hydroxymethyl)-aminomethane hydrochloric acid]. Buffer concentration was 30 - 100 mM. In most experiments the beakers were continuously aerated, for saturation with oxygen. Nitrogen was bubbled through the wastewater if low oxygen concentrations were required and pure oxygen was used if oversaturation was needed. The aeration tube was connected to a needle, that was placed near the bottom of the beaker. The bubbles were trapped before reaching the surface to prevent disturbance of the water surface. The surface area of the beakers was 87 cm². The liquid in the beakers was completely mixed due to the aeration. Sampling and analysis were the same as in the experiments in the dark environment. The intensity of the solar radiation was obtained from the Solar Energy Center (Jacob Blaustein Institute for Desert Research, Kiryat Sde Boker, Israel). Each treatment was conducted in duplicate, unless otherwise stated.

The effect of algal matter on light-mediated FC decay

The effect of algal turbidity on light-mediated FC decay was investigated by the addition of autoclaved algal matter to beakers with UASB effluent, exposed to solar radiation. Algal concentrations were 96 and 192 mg/l dry matter.

Mathematical expressions for the decay process

The FC decay is usually considered to be a first-order process, controlled by the first-order decay coefficient $K_d$ (Marais, 1974). For a batch reactor the expression is:

$$N_t / N_0 = \exp \{-K_d * t \}$$

where

$t$ = time (day)

$N_0$ = FC count at start of the experiment (#/100ml)

Table 1 Experimental conditions in the dark and solar-radiated environments

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Variable</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Aeration</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark.1</td>
<td>BOD₅</td>
<td>7.3</td>
<td>30</td>
<td>air</td>
<td>Addition of BOD₅¹</td>
</tr>
<tr>
<td>Dark.2A</td>
<td>Temperature</td>
<td>7.1-7.3</td>
<td>10, 20 or 30</td>
<td>air</td>
<td></td>
</tr>
<tr>
<td>Dark.2B</td>
<td>Temperature</td>
<td>5.5-7.0</td>
<td>10, 20 or 29</td>
<td>air</td>
<td></td>
</tr>
<tr>
<td>Dark.3A</td>
<td>pH</td>
<td>7.2, 8.3 or 9.1</td>
<td>20</td>
<td>air</td>
<td></td>
</tr>
<tr>
<td>Dark.3B</td>
<td>pH</td>
<td>7.5, 8.5 or 8.9</td>
<td>20</td>
<td>air</td>
<td></td>
</tr>
<tr>
<td>Light.1</td>
<td>Light dose</td>
<td>7.9</td>
<td>20</td>
<td>air</td>
<td>Variation in surface area exposed to radiation</td>
</tr>
<tr>
<td>Light.2</td>
<td>Temperature</td>
<td>8.1</td>
<td>20 or 30</td>
<td>air</td>
<td></td>
</tr>
<tr>
<td>Light.3A</td>
<td>pH</td>
<td>7.2, 7.9 or 8.8</td>
<td>20</td>
<td>air</td>
<td></td>
</tr>
<tr>
<td>Light.3B</td>
<td>pH</td>
<td>7.3, 8.2 or 9.0</td>
<td>20</td>
<td>air</td>
<td></td>
</tr>
<tr>
<td>Light.4A</td>
<td>DO</td>
<td>8.1</td>
<td>20</td>
<td>Nitrogen or air</td>
<td></td>
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<tr>
<td>Light.4B</td>
<td>DO</td>
<td>8.1</td>
<td>20</td>
<td>Nitrogen, air or oxygen</td>
<td></td>
</tr>
<tr>
<td>Light.5</td>
<td>Algal turbidity</td>
<td>8.1, 8.7 or 9.1</td>
<td>20</td>
<td>air</td>
<td>Various algal concentrations</td>
</tr>
</tbody>
</table>

¹ Glucose was used for the BOD₅ addition. This was done once a day, starting at the second day of the experiment. Each bottle was supplemented with 10 or 30 mg/l BOD₅ per day.
\[ N_t = \text{FC count at time } t \, (#/100\text{ml}) \]
\[ K_d = \text{first-order decay coefficient (day}^{-1}) \]

The decay coefficient \( K_d \) can be determined by fitting equation (1) to the course of the FC count in time. In some experiments the decay curve deviated from a first-order process in the last part of the experiment. In that case only data from the first part of the experiment was used for the determination of \( K_d \) (Figure 3).

Results and discussion

Dark environment experiments

Effect of BOD\(_5\) loading on light independent decay

One of the requirements for the survival of FC is the availability of carbon sources to sustain the cell metabolism. The availability of carbon sources for the bacteria was measured by the BOD\(_5\) concentration. The UASB effluent used in experiment D.1 had an initial BOD\(_5\) concentration of about 17 mg/l which decreased to about 4 mg/l BOD\(_5\) after one day of aeration. Figure 1 clearly shows that daily addition of BOD\(_5\) (glucose) to the aerated effluent reduces or entirely prevents the decay of FC. The decay of FC in the control batch was apparently the result of a lack of carbon source.

The survival of FC in aerated UASB effluent that was enriched with extra carbon source also indicates that the availability of other essential compounds, like macro- and micro-nutrients, in UASB effluent is sufficient to sustain survival for many days. The results of this experiment indicate therefore that in a dark environment very low FC decay is expected, provided that enough carbon source and nutrients are available.

The amount of BOD\(_5\) needed for survival is probably around 10 (mg BOD\(_5\))/(litre.day). This equals the volumetric BOD\(_5\) loading of, for instance, a 1.5 m deep facultative pond with a surface loading of 150 kg BOD\(_5\)/(ha.day). In such a pond FC decay in the dark parts may be very low.

Effect of temperature on light-independent decay

It was found that the decay of FC in aerated UASB effluent (i.e. conditions of carbon shortage) increased with increasing temperatures in the range 10°C to 30°C (Figure 2). This may be explained by the positive effect of temperature on the rate of cell metabolism. The consumption of carbon by the micro-organisms in UASB effluent is therefore increasing with
temperature and a situation of carbon shortage is aggravated (Pearson et al. 1987). The removal or decay of FC in stabilization ponds is affected by the temperature of the pond water (Marais, 1974). The expression proposed by Marais (1974) is most widely applied and models the temperature effect on FC decay in stabilization ponds with an Arrhenius type equation: \( K_d = 2.6 \times (1.19)^{T-20} \) (2 < T < 21°C), where T is the temperature (°C). The expression derived from current batch experiments (Fig. 2b) \( K_d = 0.06 \times T - 0.54 ; R^2 = 0.74 \) predicts much lower values, obviously due to the exclusion of light-mediated decay processes.

**Effect of pH on light-independent decay**

The effect of alkaline pH values were investigated by incubating UASB effluent in the dark at pH values in the range 7.2 - 9.1. During the first day of incubation, growth was observed at all pH values. This indicates that growth of FC is possible at alkaline pH values up to 9.1. The FC are apparently able to maintain their homeostasis with respect to the internal cytoplasmatic pH. FC decay started after the initial growth, but the decay rate was not affected by the pH in the applied range. The decay under those conditions was probably caused by a shortage in carbon source. This shortage was apparently not affected by the pH values applied. The results are in accordance with the findings of Parhad and Rao (1974) who found that FC were able to grow in media with pH values of 9.2 and lower. They observed, however, that pH values above this critical value caused a rapid decay. Such high pH values have a direct effect on FC survival, for instance by destruction of enzymes or destabilization of the internal cytoplasmatic pH. Curtis et al. (1992a, b) also found that pH values below a critical pH of 9.3 hardly or not at all influenced the decay of FC in a dark environment.

**Experiments in a solar-radiated environment**

*Effect of “light input” on light-mediated decay*

In a solar-radiated pond environment decay of FC not only occurs due to a shortage of carbon source, but also due to light-mediated processes. Those processes are likely to be affected by the radiation intensity. Since it was practically difficult to control the radiation intensity that reaches the experimental beakers, it was assumed that the reduction of the exposed open surface area can closely simulate the reduction of the solar-radiation intensity. This assumption can be supported also by the complete mixing in the beaker. Therefore, the term “light input” will be used herein instead of light intensity. A shown in Figure 3, the
decay rate decreased with decreasing light input. The curves for 56\% and 100\% light input show a clear deviation from a first-order decay fit. The first-order decay coefficient was calculated however from the slope of the curves during the period which fits first-order decay rate. The following expression was derived: $K_d = 20.7 + 0.0623 \times I_r$ ($R^2=0.79$), where $I_r$ is the radiation intensity (W/m$^2$).

**Effect of temperature on light-mediated decay**

The decay rates caused by solar exposure are much higher than the decay rates obtained in a dark environment. The relevance of the light-independent processes to the overall decay in radiation exposed beakers is therefore of minor or negligible importance. The environmental factors that affect the solar independent decay processes, for instance temperature, are therefore not necessarily important for the total decay under solar radiation. An experiment with beakers exposed to solar radiation at 20\$'$C and 30\$'$C showed that the temperature did not affect the decay under the applied conditions. The light-mediated decay processes (UV adsorption and photo-oxidation) are apparently not affected by temperature, as was also found by Kapuscinski and McDonald (1980) and Curtis _et al._ (1992a). However, FC decay in stabilization ponds was shown to be dependent on temperature (Marais, 1974). This is most probably caused by temperature effects on biological processes, like algal photosynthesis, that indirectly affects the light-mediated decay process (see below).

**Effect of pH and DO concentration**

The photo-oxidation process is affected by the pH and the DO of the wastewater. The current experiments show a linear increase of the decay coefficient with both pH and DO (Fig. 4), as was reported previously for maturation pond water by Curtis _et al._ (1992a, b). However, the expressions found for samples of UASB effluents collected at different dates were quite different. The composition of the UASB effluents fluctuated extremely. The FC decay rate was consequently fluctuating too, even under the same environmental conditions.

The combined effect of solar radiation, pH and DO on light-mediated decay

Development of one expression that predicts the combined effect of pH, DO and radiation, on the basis of the current experimental results is very difficult. This is due to the different

![Figure 3](https://iwaponline.com/wst/article-pdf/42/10-11/111/427985/111.pdf)

**Figure 3** FC counts in beakers with UASB effluent exposed to solar radiation (around 1000 W/m$^2$), with surface area open to solar radiation 10, 56 and 24\% respectively (Exp. L.1: 16 June 1997; sunrise 6.00 a.m.; sunset 19.30 a.m.; experiment start 10.17 a.m.; oxygen saturated conditions; 20\$'$C , pH = 8; all data from duplicate beakers)
expressions that were found for duplicated experiments with different effluent samples (Figure 4). Regression analysis of the data is therefore not reported here. A mathematical expression for the combined effect of radiation, pH and DO is given by Curtis et al. (1992a) for the decay in similar batch experiments. The $K_d$ values calculated by that equation for our beaker experiments were lower than those measured (approximately 40% difference).

Effect of algal growth on FC decay in stabilization ponds

Algal growth affects the FC decay in stabilization ponds in two ways. Firstly, algal material and other particles absorb solar radiation in the upper layer of a stabilization pond. Therefore, only in the upper layer light mediated decay occurs. Secondly, algal photosynthesis raises the pH and DO, thus stimulating the photo-oxidation process. A mathematical expression taking into account solar radiation intensity, light attenuation by algal matter, pH and DO, could predict the optimum combination of those factors for FC decay. The experiment described in Figure 5 illustrates the complexity of this issue. It shows that the positive effect on FC decay that is expected from a pH rise, was completely compensated for by an increased light attenuation by algal matter.

**Figure 4** The first-order decay coefficient $K_d$ as a function of pH (a) and dissolved oxygen concentration (b) general experimental conditions: solar radiation 900-1000 W/m²; 20°C; sunrise 6.00 a.m.; sunset 18.20 p.m. experiment start 10.00 a.m.; all data from duplicate beakers; for a) DO = 7.5 and for b) pH=8.1)

**Figure 5** FC counts in batches with UASB effluent under solar radiation, at various pH and algae concentrations (Exp. L.5: 31 August 1997; oxygen saturated conditions; 20°C; data for the beakers with algae matter is from duplicate beakers, while the data for the control is from a single beaker)
Conclusions

The decay of FC in aerated UASB effluent in darkness was found to be the result of a shortage of carbon source. Addition of carbon source (10 mg/(litre.day) glucose) prevented decay for more than 10 days. Macro- and micro-nutrients were sufficiently present in the UASB effluent for prolonged FC survival. The carbon shortage and therefore the decay was shown to increase linearly with temperature. The pH in the range of 7.2 to 9.1 did not affect the FC decay process in the dark. The FC decay process in the dark parts of a stabilization pond with satisfactory nutrient content is therefore determined by the availability of an organic carbon source (BOD$_5$ loading) and the temperature.

The FC decay rate in beakers exposed to solar radiation was much faster than in a dark environment. The light mediated decay was found to be affected by the pH, the oxygen concentration, the solar intensity and by the light attenuation. Mathematical expressions for the relationships between each single environmental factor and the first-order decay coefficient, were derived for light mediated decay. Developing one expression that predicts the combined effect of pH, DO and solar intensity could not be done, due to insufficient data and the large fluctuations of the decay rate in UASB effluent, under similar environmental conditions. The temperature (20-30 °C) did not influence the light mediated decay.

The importance of light attenuation in light-mediated decay processes was demonstrated by addition of algae matter to the UASB effluent. The light attenuation by 100 mg/l dry matter prevented almost entirely the decay. Light attenuation by algae apparently partially compensates the stimulating effects of algae photosynthesis (increased DO and pH) on photo-oxidation. The two contradicting effects of algae on FC decay suggest that there is an optimum algae concentration with respect to FC decay. A new approach to stabilization pond design could therefore include measures to regulate algae concentration for maximum FC removal.

References


