# Development of an empirical mathematical model for describing and optimizing the hygiene potential of a thermophilic anaerobic bioreactor treating faeces

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**Abstract** Poor sanitation and insufficient disposal of sewage and faeces are primarily responsible for water associated health problems in developing countries. Domestic sewage and faeces are prevalently discharged into surface waters which are used by the inhabitants as a source for drinking water. This paper presents a decentralized anaerobic process technique for handling of such domestic organic waste. Such an efficient and compact system for treating faeces and food waste may be of great benefit for developing countries. Besides a stable biogas production for energy generation, the reduction of bacterial pathogens is of particular importance. In our research we investigated the removal capacity of the reactor concerning pathogens, which has been operated under thermophilic conditions. Faecal coliforms and intestinal enterococci have been detected as indicator organisms for bacterial pathogens. By the multiple regression analysis technique an empirical mathematical model has been developed. The model shows a high correlation between removal efficiency and both, hydraulic retention time (HRT) and temperature. By this model an optimized HRT for defined bacterial pathogens effluent standards can be easily calculated. Thus, hygiene potential can be evaluated along with economic aspects. In this paper not only results for describing the hygiene potential of a thermophilic anaerobic bioreactor are presented, but also an exemplary method to draw the right conclusions out of biological tests with the aid of mathematical tools.
**Keywords** Bacterial pathogens; decentralized sanitation and reuse; faeces; multiple regression analysis; thermophilic anaerobic technology

## Introduction

The International Drinking Water Supply and Sanitation Decade (1981–1990) and the Safe Water Supply Decade (1991–2000) with the goal to supply the population world-wide with drinking water and sanitation have just been finished. Up to the present, a significant part of the world population still has no access to clean potable water and suitable sanitation. Target 10 of the Millennium Development Goals demands to halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation (http://ddp-ext.worldbank.org). Concerning wastewater treatment, Asia has the biggest requirements for sanitation due to an increase in people to be supplied of about 320,000 per day, followed by Africa with 91,000 and Latin America with 32,000 (WHO, 2000; Mara and Faechem, 2001). In order to fulfil the goal "water and sanitation for all" alternatives have to be searched for, apart from the "classical" wastewater treatment systems consisting of a water-borne sewage system and a central wastewater treatment plant. An interesting alternative could be the DESAR-concept (Decentralized Sanitation and Reuse). A decentralized anaerobic process technique for treating domestic organic waste is presented in this work. Faecal sludge as the solid part of the single domestic wastewater streams is treated in an anaerobic bioreactor to produce energy by degrading

organic compounds and to regain nutrients. The effluent is intended to be used for agricultural purposes like irrigation.

Anaerobic bioreactors as decentralized wastewater treatment systems are, especially for rural areas, economically and ecologically reasonable techniques. The main objective of applying thermophilic process conditions is the removal of pathogens and not an additional reduction of the organic compounds. If the effluent is used for irrigation, removal of pathogenic organisms is one of the most important tasks. Many pathogenic germs are excreted with faeces and so reach waterbodies, where they get access to the water cycle. Along with these epidemic germs, intestinal bacteria are also excreted in a high amount. These intestinal bacteria don't represent a hazard for consumers, but represent an indication that epidemic germs (in a much smaller amount) are also present.

In this work, intestinal enterococci and faecal coliforms have been detected as indicator organisms for bacterial pathogens to evaluate the hygiene potential of the thermophilic anaerobic reactor. Although the detection of these organisms is quite simple, the ongoing determination in the laboratory is time consuming. The overall objective was to develop a mathematical model, which can describe the reduction of the bacterial indicator organisms. Knowing the effluent contamination for different operational conditions and reducing laboratory effort can strongly enhance the benefit of the investigated decentralized anaerobic process technique.

## Methodology

The anaerobic bioreactor (Figure 1) has been operated under thermophilic conditions (55 °C). The volume of 500 litres was separated into an outer chamber for hydrolysis and



**Figure 1** Thermophilic anaerobic bioreactor

an inner part for methanogenesis. The experiments were started in October 2004 with faeces as sole substrate and were finished in March 2005.

### Sampling and processing

Samples were collected from the inlet and outlet of the reactor once a week. Raw influent samples (0.5 litres) were collected in clean sampling bottles, transported to the laboratory and processed on the same day. Detection and enumeration of faecal coliforms was done in test tubes fitted with Durham tubes according to the "Most-Probable-Number-Process" (MPN-Process). Fluorocult-Lauryl-Sulfate-Bouillon (Merck 12588) was used as culture medium. The tubes were inoculated with 1 ml of faecal sludge and incubated for $(48 \pm 3)$ hours at $(37 \pm 1)$ °C. The presence of faecal coliforms is indicated by fluorescence under a long wavelength UV lamp (360 nm). The enumeration of the bacterial count was carried out with the aid of the "McCrady table" (deMan, 1983). The results are referred to 1 ml sample volume [MPN/1 ml]. Detection and enumeration of intestinal enterococci was performed according to ISO 7899-2. Cellulose nitrate filters with a pore size of 0.45 µm were placed on an enterococcus selective agar according to Slanetz and Bartley (1957) and inoculated for $(48 \pm 3)$ hours at $(44 \pm 2)$ °C. Pink to brown colonies with a diameter of 0.5 to 2 mm are usually enterococci. If characteristic colonies were detected, the filter was placed to a Bile Esculin Azide Agar and inoculated for 2 hours at $(44 \pm 0.5)$ °C. All colonies from tawny to black color were enumerated.

### Multiple regression analysis

Simple regression and correlation are used when there are two variables multiple regression is used to express the relationship between one variable and two or more other variables. In general, regression is useful when the relationship between variables is of functional dependence. If so, the magnitude of one variable called the dependent variable, is assumed to be determined by the other variables, called the independent variables. When there is only one independent variable in addition to the dependent variable, the term simple regression is used. When there are two or more independent variables, the term multiple regression is used. To investigate the relationship between the independent variables $x$ and the dependent variables $y$, a model of the form

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_p x_{pi} + \varepsilon_i \quad i = 1, 2, \ldots n$$

can be used, where $\beta_0, \beta_1, \ldots, \beta_p$ are called regression coefficients. Because there is a unique value $\varepsilon_i$ corresponding to each value of $y_i$, the $n$ data points will not all lie on a line (They would if $\varepsilon_i$ was always zero). The $n$ data points can be used, however, to estimate $\beta_0, \beta_1, \ldots, \beta_p$. The $\beta$ are calculated using the method of least squares, which means to minimize the sum of the squared $\varepsilon$. This can be expressed by the following equation

$$S(\beta_0, \beta_1, \beta_2, \ldots, \beta_p) = \sum_{i=1}^{n} \varepsilon_i^2 = \sum_{i=1}^{n} (y_i - \beta_0 - \beta_1 x_{1i} - \ldots - \beta_p x_{pi})^2$$

### Computing software

Multiple regression analysis as a complex mathematical method makes high demands on the computing software used. For multiple regression analysis only carefully tested professional mathematical software packages should be used. In our research we used *Mathematica* in its current version 5.0. *Mathematica*, first released in 1988 by Wolfram Research, Inc., combines symbolic manipulation, numerical mathematics, outstanding graphics, and a sophisticated programming language.

### Results and discussion

The removal of faecal bacteria in anaerobic bioreactors depends on several mechanisms influenced by both, time and environmental conditions. The main factors in our research which have been found to have significant effects on faecal bacteria removal are the hydraulic retention time and the temperature within the reactor. During the investigation period the influent to the reactor was successively raised to gain information about removal efficiency under different HRT. Figure 2 and Figure 3 show the removal efficiency for intestinal enterococci and faecal coliforms depending on the hydraulic retention time.

The thermophilic reactor should reach high removal efficiencies ($E > 99.9$), so that the effluent can be used for irrigation. Both figures demonstrate that the hydraulic regime of the reactor has a great influence on the removal efficiency of the indicators of faecal contamination. An efficiency $E = 90\%$ corresponds to the removal of one logarithmic unit, an efficiency $E = 99.9999$ corresponds to the removal of 6 log units, according to equation 1.

$$removed \log units = -\log_{10}\left(\frac{100 - E}{100}\right) \qquad (1)$$

If the effluent is to be used for unrestricted irrigation, the World Health Organization (WHO, 1989) recommends values of less than 1000 faecal coliforms per 100 ml. Considering that the faecal coliforms count in the raw influent is in the order from $10^6$ to $10^9$ per 100 ml, the removal efficiency of the reactor should be very high. To comply with the WHO criteria, faecal coliforms removal efficiency in the order of 3 to 5 log units (99.9 to 99.999) are necessary to be reached.

To obtain efficiencies higher than 99.9% (3 log units removed) for faecal coliforms removal, the hydraulic retention time has to be above 2 days. The same efficiency for intestinal enterococci can be reached by a hydraulic retention time above three days. A hydraulic retention time above 2.5 days showed to be sufficient for a complete reduction of faecal coliforms. Intestinal enterococci, however, couldn't be removed completely, but were always less than 10/ml when a HRT of 5 days was applied. To remove at least two log units of both intestinal enterococci and faecal coliforms, the HRT must be higher than 1.5 days.
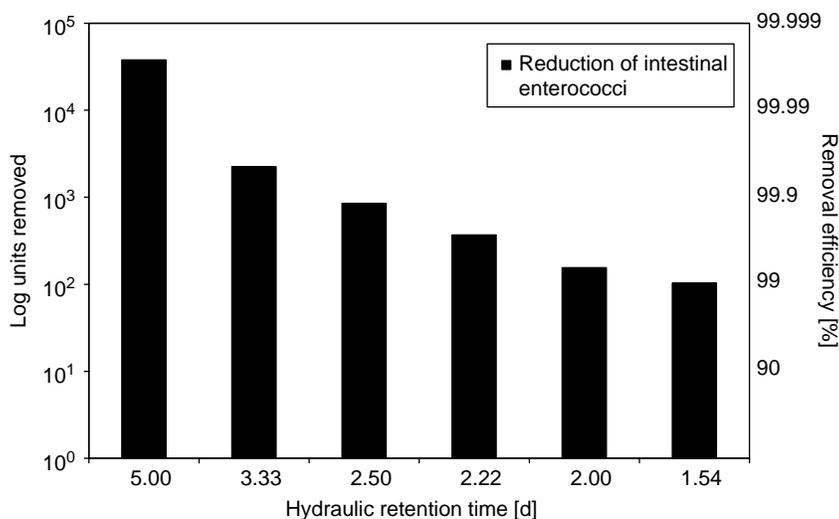


**Figure 2** Removal efficiency of intestinal enterococci depending on the hydraulic retention time
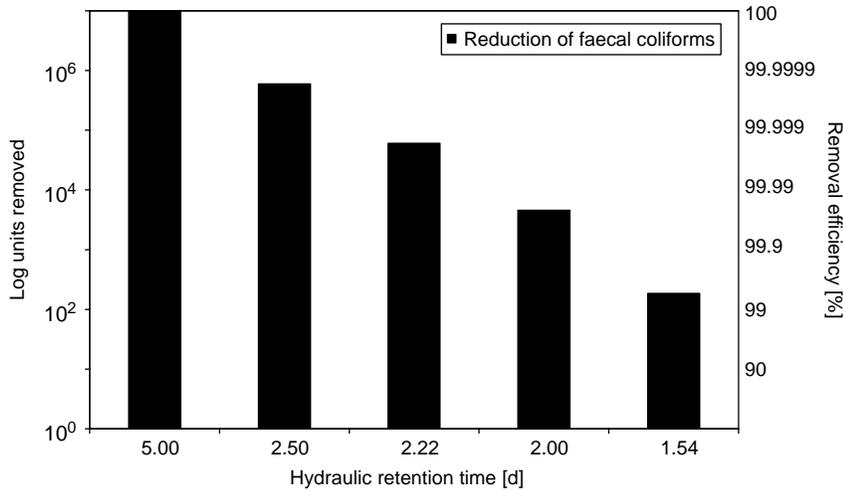
**Figure 3** Removal efficiency of faecal coliforms depending on the hydraulic retention time

For more detailed information about the optimal hydraulic retention time, a regression term can be derived describing removal efficiency in terms of hydraulic retention time. Besides the hydraulic retention time, however, temperature is the main factor influencing removal efficiency. Figure 2 and Figure 3 show results only under constant thermophilic conditions (55 °C). A regression term is useless if the temperature hasn't been taken into account.

Temperature drop as a result of power failure delivered valuable information about removal efficiency under different temperature conditions. Using multiple regression analysis, intestinal enterococci and faecal coliforms removal can be represented in terms of HRT and temperature by the following equations:

Equation 2 shows the multiple regression term for intestinal enterococci removal.

$$\eta_{IE} = 98.29 - 2.2\left(\frac{1}{HRT}\right)^2 + 0.031T \tag{2}$$

$\eta_{IE}$ = intestinal enterococci removal [%]
HRT = hydraulic retention time [d]
T = temperature [°C]

Equation 3 shows the multiple regression term for faecal coliforms removal.

$$\eta_{FC} = 98.29 - 1\left(\frac{1}{HRT}\right)^2 + 0.031T \tag{3}$$

$\eta_{FC}$ = faecal coliforms removal [%]
HRT = hydraulic retention time [d]
T = temperature [°C]

Equation 2 has a correlation coefficient of 0.93 with $p = 0$. Equation 3 has a correlation coefficient of 0.99 with $p = 0$. This shows that the removal efficiency corresponds very well with the hydraulic retention time and the temperature.

Figure 4 depicts the comparison between measured and modelled reduction of intestinal enterococci, Figure 5 for faecal coliforms.

Both figures demonstrate a good concordance between measured and modelled removal efficiencies. According to the Equations 2 and 3 and as demonstrated in Figures 4 and 5, removal of intestinal enterococci is more sensitive to the applied hydraulic
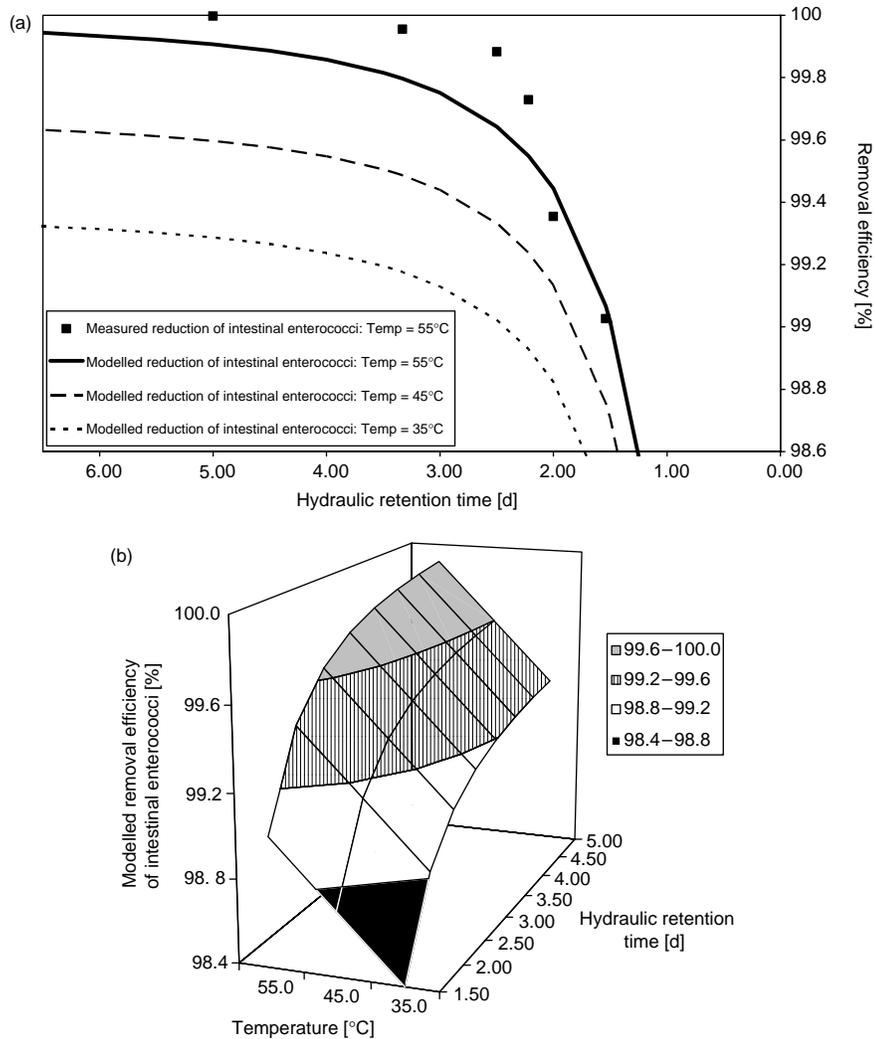
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**Figure 4** Modelled and measured removal efficiencies of intestinal enterococci in terms of hydraulic retention time and temperature: (a) modelled and measured, (b) only modelled shown as surface graphic

retention time than for faecal coliforms. Both models show the same linear influence of temperature, but the coefficient describing the influence of HRT is 2.2 times higher for intestinal enterococci than for faecal coliforms. As indicated in Figure 4 and Figure 5, the lower the HRT the higher the influence of HRT on the achievable removal efficiency. At higher HRT temperature is the main factor for increasing removal efficiency. Figure 4 explains that until a HRT of 2 days a stepwise increase of 0.5 days regarding the HRT is more efficient in intestinal enterococci removal than increasing temperature by 10 °C. Above 2 days, raising the temperature is more efficient. Referring to faecal coliforms, the same threshold can be set to 1.5 days.

The area of validity of both models is the range of data used for regression analysis. In praxis, the HRT varied between 1.5 and 5 days, the temperature between 35 and 55 °C. It is not possible to widely expand the models out of the data ranges from which the models have been derived (Draper and Smith, 1998). However, the range of data is expanded enough to give valuable information about an optimized HRT in case of an effluent standard of interest.
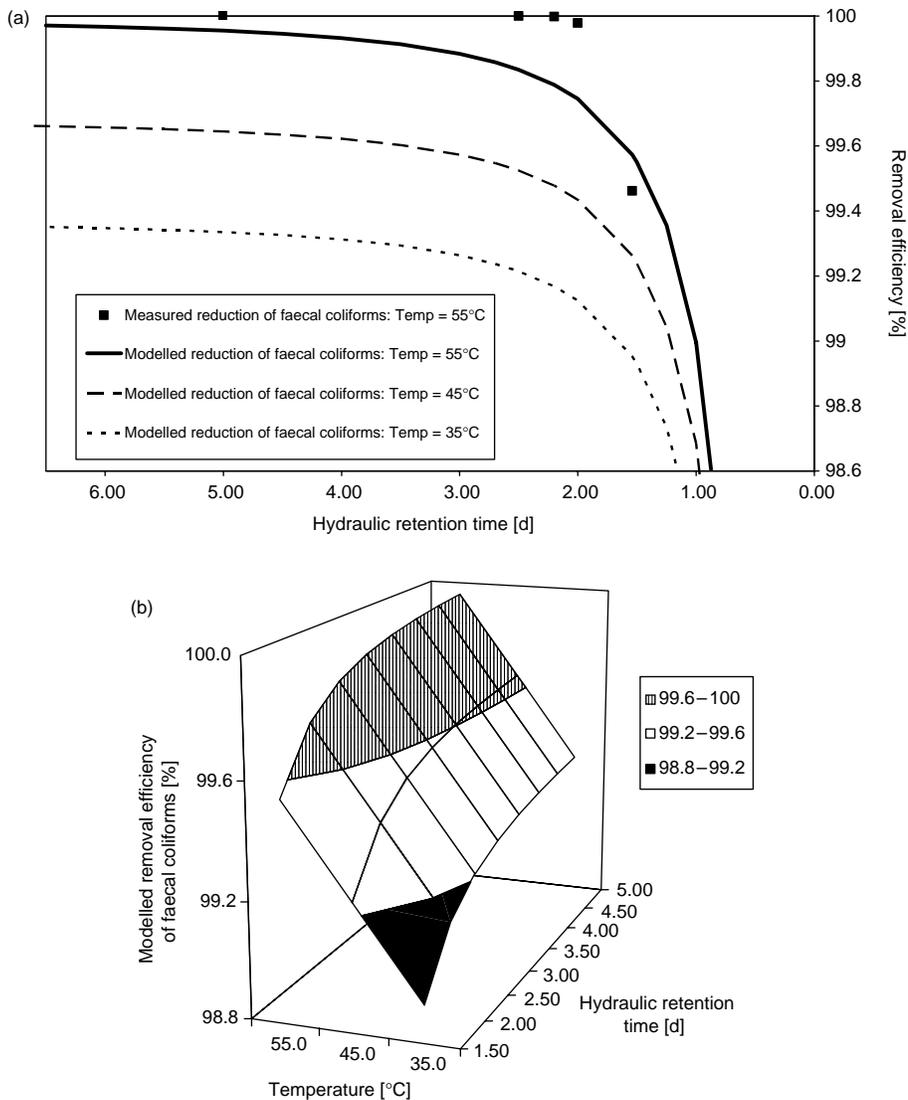
**Figure 5** Modelled and measured removal efficiencies of faecal coliforms in terms of hydraulic retention time and temperature: (a) modelled and measured, (b) only modelled shown as surface graphic

## Conclusions

Results obtained in our study indicated that both HRT and temperature have the most significant effect on pathogen removal, measured in terms of faecal coliforms and intestinal enterococci numbers. The derived models indicated that intestinal enterococci removal is more sensitive to the applied HRT than faecal coliforms removal. Secondarily, the models show an identical linear influence of temperature. Until a threshold of 2 days for intestinal enterococci and 1.5 days for faecal coliforms, increasing HRT by 0.5 days is more efficient than increasing temperature by 10 °C.

The models represent a mathematical tool to optimize both, hygiene potential and operation, for different effluent standards. The derived empirical models are expected to apply to faecal sludge treatment in decentralized thermophilic anaerobic bioreactors. The objective is to give planners a mathematical tool to optimize hygiene potential and operation for different effluent standards.

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