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Reduction of the nitrogen and carbon content in swine waste with algae and bacteria

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Abstract Animal waste causes environmental problems like eutrophication of ground and surface water or the pollution of the atmosphere because of its high NH_4^+ content. The aim of our study was to fix the nitrogen of swine waste as biomass. Therefore, an isolated alga, *Chlorella* sp., and bacteria naturally living in liquid manure were grown in batch cultures (containing diluted swine waste supplied with a nutrient solution) and continuous cultures (undiluted liquid manure) to achieve reduction of NH_4^+ and total organic carbon (TOC) contents. For continuous cultivation, a photobioreactor of our own design was used. The batch cultivation of *Chlorella* sp. and bacteria in swine waste resulted in good growth of both groups of organisms and in a reduction of 25% NH_4^+ and 80% TOC. In the continuous cultivation a steady state was not achieved owing to a change in the composition of the bacterial population. NH_4^+ was totally removed, but NO_2^- (up to 100 mM) was transiently released. NO_3^- was not detected. These effects might be explained by the presence of heterotrophic nitrifiers, which are able to oxidize NH_4^+ to NO_2^- and to reduce NO_2^- to gaseous compounds.

Introduction

The increase in intensive animal production during the last decades has resulted in an excess of animal waste. The disposal of animal waste onto land causes a lot of environmental problems (Kirchgessner and Roth 1991; Pfeffer 1992), many of which are due to the high nitrogen content of animal waste (180–320 mM NH_4^+). Up to 70% of the nitrogen present in liquid manure is ammonium. During the spreading of swine waste onto land, up to 80% of the ammonium can be released into the atmosphere (Hoffmann and Hege 1987) as toxic ammonia emissions (Archer and Nicholson 1992; Verstegen et al. 1994).

One solution to reduce the environmental problems that derive from animal waste nitrogen is the fixation of this nitrogen as biomass and utilization of the resulting product as fertilizer, avoiding ammonia emissions. Suitable organisms for a cultivation in animal waste are algae, because they are able to grow autotrophically at the low C/N ratio of animal waste. Outdoor ponds are used for the cultivation of algae (Fallowfield and Garrett 1985; Oswald 1988; Richmond 1992; Chaumont 1993), sunlight being the energy source. However, it is difficult to regulate their pH value and temperature; therefore, in many regions of the world, climatic conditions do not allow the use of algal ponds.

In our study we used a photobioreactor of our own design for the cultivation of a mixed population containing algae and bacteria. The algae are able to produce oxygen and metabolites that can be utilized by the bacteria. The photobioreactor was constructed in such a way that the algal culture was supplied with high light intensities. The photobioreactor was used indoors, which allows controlled illumination, pH control and temperature regulation. We investigated the N and C uptake of the algae/bacteria population in batch and continuous cultivations in diluted swine waste.

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Materials and methods

Algae

The photobioreactor was used with the following medium to isolate a suitable alga that can grow in the presence of high NH_4^+

concentrations: 70 mM NH_4Cl, 0.20 mM $MgSO_4 \cdot 7H_2O$, 0.10 mM $CaCl_2 \cdot 2H_2O$, 1.6 μM H_3BO_3, 0.35 μM $ZnSO_4 \cdot 7H_2O$, 0.1 μM $MnSO_4 \cdot H_2O$, 0.041 μM $NaMoO_4 \cdot 2H_2O$, 0.34 μM $Co(NO_3)_2 \cdot 7H_2O$, 0.20 μM $CuSO_4 \cdot 5H_2O$, 40 mM KH_2PO_4/Na_2HPO_4 buffer (pH = 6.5), 1 ml FeEDTA (see Medium with swine waste), 0.2 mg l^{-1} vitamin B_1, 0.02 mg l^{-1} vitamin B_{12}.

A population of algae grew in the photobioreactor with non-sterile cultivation after 7 days of aeration. The algae were plated on tryptone/soya/agar (Oxoid, Wesel) and illuminated by artificial daylight at room temperature to receive a sterile culture of algae.

Bacteria

Bacteria were not inoculated. During the non-sterile cultivation the indigenous population grew in liquid manure.

Medium with swine waste

Samples of swine waste were collected under the slatted floor of a fattening house and stored at −20 °C until used. Prior to utilization, the waste was centrifuged at 3000 *g* for 3 min (Cryofuge 8000; Heraeus, Hanau, Germany). For preparation of the medium the supernatant was diluted with tap water to give a final concentration of 10% liquid manure and supplemented with the following minerals (final concentrations): 1 mM $MgSO_4 \cdot 7H_2O$, 100 mM KH_2PO_4/Na_2HPO_4 buffer (pH = 6.5); trace metals (Schlösser 1982): 1.0 μM H_3BO_3, 1.0 μM $MnSO_4 \cdot H_2O$, 1.0 μM $ZnSO_4 \cdot 7H_2O$, 0.016 μM $CuSO_4 \cdot 5H_2O$, 0.010 μM $(NH_4)_6Mo_7O_{24} \cdot H_2O$, 25 μM $FeSO_4 \cdot 7H_2O$, 25 μM Na_2EDTA. During cultivation the pH of the medium was maintained at 6.5 with 1 M HCl to prevent ammonia emissions.

Batch cultivation

For batch cultivation 450 ml medium containing 10% swine waste in a 1-l conical flask was inoculated with 50 ml algal suspension (7 days old) with the following concentrations of minerals: 28 mM NH_4Cl, 1 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM $CaCl_2 \cdot 2H_2O$, 25 mM KH_2PO_4/Na_2HPO_4 buffer (pH = 6.5) (for concentrations of trace metals see Medium with swine waste.) The culture was aerated at a flow rate of 130 l h^{-1}. To reduce evaporation the air was bubbled through a washing flask containing distilled H_2O. The culture was illuminated for 14 h/day with 7000 lx (three L58/W31 lamps and two L58/W11 lamps; Osram, München) at room temperature (20 ± 2 °C). The pH of the culture was controlled with a pH electrode (Mettler Toledo Typ E 66; Gießen, Germany) and adjusted to pH 6.5 with 3 M HCl or 3 M NaOH.

Continuous cultivation

The algal/bacterial mixed culture was grown in the photobioreactor (about 10 l) shown in Fig. 1.

Undiluted liquid manure was supplied at a flow rate of 240 ml day^{-1}. The culture was illuminated with five lamps (three L58/W31 und two L58/W11; Osram, München, Germany) to obtain 3000–7000 lx on the collector surface. The pH was measured with a pH electrode (Mettler Toledo Typ E 66; Gießen, Germany) and adjusted to 6.8 (fermentor HT ISF-200, Infors; Einsbach, Germany) with 3 M HCl or 3 M NaOH. The temperature was measured and adjusted to 20 °C with a heating staff (Hydromatic T 150 Vitakraft; Bremen, Germany). O_2 was measured with an electrode (OXI 91 WTW; Weilheim, Germany) and regulated with the air inlet to over 90% saturation.

Analyses

The number of algae was determined by microscopic (magnification: 300×) counting of suitable dilutions (in tap water), using a Thoma chamber (Brand, Wertheim). The number of viable bacteria was determined by plating decimal dilutions (in 0.9% NaCl; 0.1% Trypton, Difco) on tryptone/soya/agar (Oxoid, Wesel) also containing 0.2% yeast extract (Difco). The plates were incubated aerobically for 3 days at 30 °C.

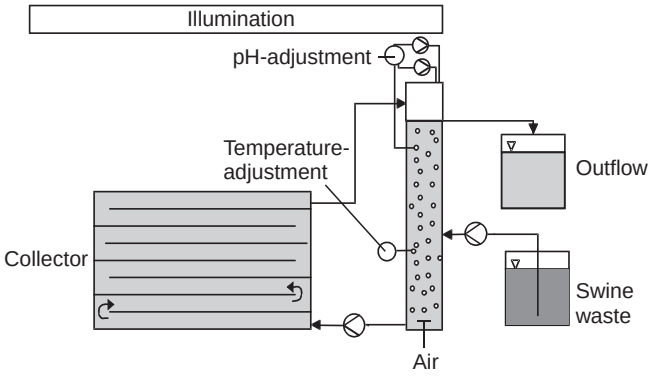

Fig. 1 Schematic representation of the photobioreactor

NH_4^+ was determined volumetrically after distillation (equipment employed: Distillation Unit 321, Büchi, Konstanz, Germany; Dosimat 665, Impulsomat 614, Methrom; pH electrode 405-S7, Ingold, Microprocessor pMX 2000, WTW).

The NO_2^- was determined, as described in European norm 26777, by a PU 8740 UV/VIS scanning spectrophotometer (Philips, Hamburg) or as described by Claus and Berkeley (1986).

NO_3^- was determined after reduction to NO_2^- with Zn powder. NO_2^- was determined qualitatively as described by Claus and Berkeley (1986).

Total organic carbon (TOC) was determined with a TOC 5050 and ASI 5000 analyser (Shimadzu, Duisburg, Germany). Protein was determined as described by Bradford (1976).

Results

The isolated alga was identified to be *Chlorella* sp. and was used for the following cultivations.

Batch cultivation

Figure 2 shows a batch cultivation of *Chlorella* sp. and the developing bacterial population in swine waste.

During this experiment the algae exhibited a lag phase of 4 days, followed by exponential growth for 8 days, and reached 2×10^8 cells ml^{-1} after 24 days. The bacteria started growth immediately without a lag phase and the final concentration was 8×10^8 cells ml^{-1}, after 4 days. Subsequently, the cell concentration decreased.

The ammonium content of the medium decreased from 30 mM to about 23 mM after 3 days, increased to about 25 mM during the next 5 days and subsequently decreased slowly to 14 mM during the last 10 days. The TOC decreased during 2 days from 900 mg l^{-1} to about 200 mg l^{-1}, and remained constant.

Continuous cultivation

Figure 3 shows a continuous cultivation of the isolated *Chlorella* sp. and the developing bacterial population in

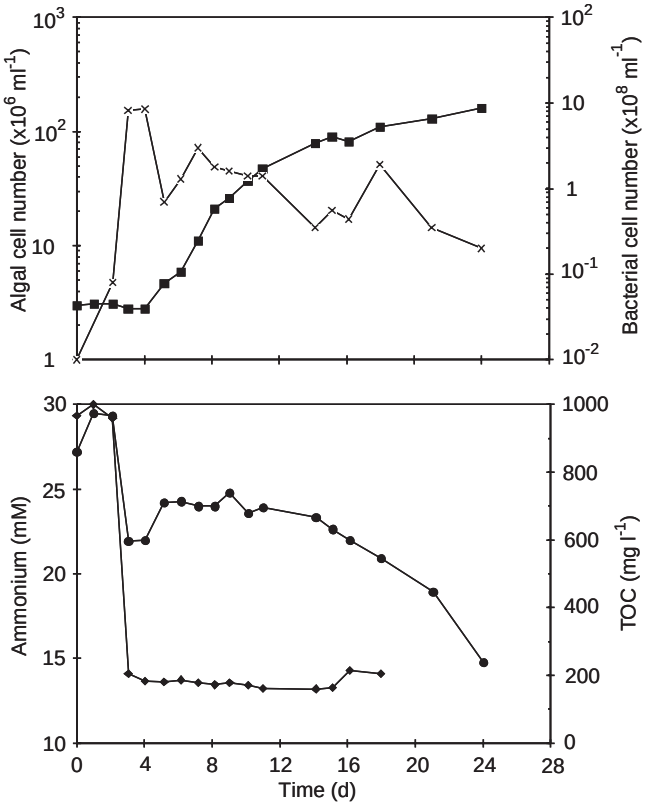

Fig. 2 Batch cultivation of *Chlorella* sp. in diluted swine waste (■ algae; × bacteria; ● ammonium; ◆ total organic carbon TOC)

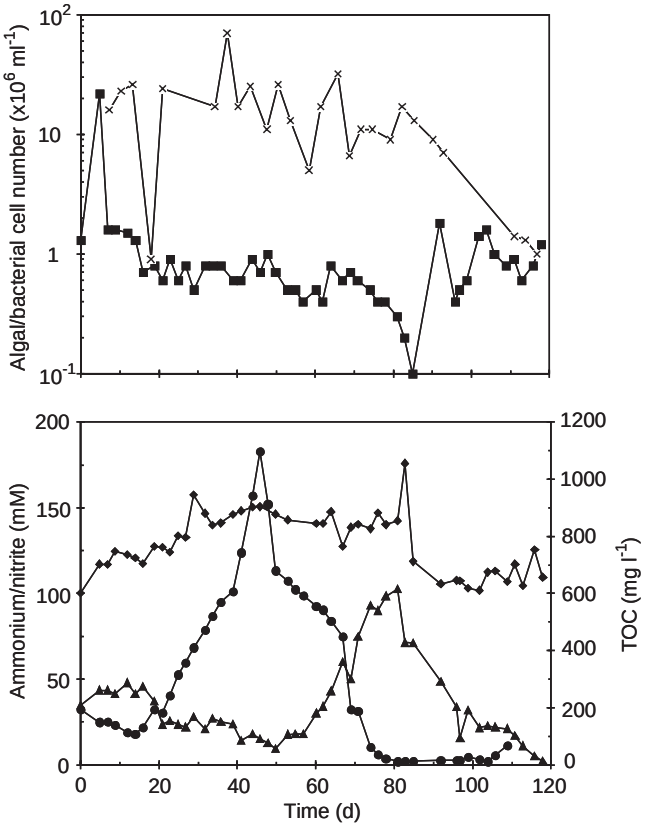

Fig. 3 Continuous cultivation of *Chlorella* sp. in the photobioreactor with swine waste over a longer period (■ algae; × bacteria; ● ammonium; ◆ total organic carbon TOC; ▲ nitrite)

the laboratory-built photobioreactor with undiluted liquid manure.

During this cultivation the algal cell number decreased slowly from 10^6 ml^{-1} to 10^5 cells ml^{-1} during 85 days and subsequently increased to 10^6 cells ml^{-1} again. The bacterial cell number varied considerably (about 1×10^7–3×10^7 ml^{-1}) and decreased during 80 days to 10^6 ml^{-1}. In addition the bacterial population changed its composition. On agar plates, for the determination of viable cell numbers, an increasing number of slow-growing and small bacterial colonies were observed, which needed a 7-day incubation before becoming visible. The NH_4^+ content in the continuous cultivation increased through the addition of undiluted swine waste to the photobioreactor. After day 50, the NH_4^+ content decreased and the NO_2^- concentration rose to 100 mM, but NO_3^- was never detected. During the following 40 days, NO_2^- was totally consumed. The TOC ranged from 600 mg l^{-1} to 900 mg l^{-1} with no significant increase or decrease.

Discussion

Batch cultivation

The mixed algal and bacterial population showed good growth and NH_4^+ as well as TOC depletion in batch cultivation (Fig. 2) in spite of algae (eukaryotes) and bacteria (prokaryotes) having different lag phases and growth rates. Therefore, it is possible to establish a co-cultivation of algae and bacteria. The decrease of NH_4^+ and TOC concentration on the third day correlated with the exponential growth phase of the bacteria, suggesting that depletion of NH_4^+ and TOC in the medium was not a result of algal growth but of bacterial growth.

Continuous cultivation

During continuous cultivation, the growth rate of *Chlorella* sp. and the bacterial cell number decreased considerably (Fig. 3). This was not a washing-out effect, because the inflow of swine waste was very low (240 ml day^{-1}). This decrease can be explained by the following:

1. Algae and bacteria inhibit each other if cultivated over a longer period. The antibacterial activities of algae (Debro and Ward 1979; Pesando 1990) and antialgal activities of bacteria (Dor and Svi 1980; Hayashida et al. 1991; Dakhama et al. 1993) have already been described.
2. Liquid manure often contains high concentrations of heavy metals (Wilcke and Döhler 1995), which may have toxic effects after adsorption to or absorption by cells (Maeda and Sakaguchi 1990; Fehrmann and Pohl 1993). Thus, the continual inflow of liquid manure led to an enrichment of heavy metals in or on the cells.

In addition, the high accumulation of nitrite produced in the photobioreactor may be an explanation for the low number of algal and bacterial cells. A batch cultivation with *Chlorella* sp. in mineral medium with 110 mM NO_2^- (found in the continuous cultivation) revealed no growth (data not shown). However, it seems unlikely that the high NO_2^- concentration was the only reason for the decreasing algal cell number. The formation of nitrite may also have led to the reduction of fast-growing bacteria. A population of slowly growing NH_4^+-oxidizing bacteria established itself in the photobioreactor that were difficult to isolate on tryptone/soya/agar (see Materials and methods). Such bacteria oxidize NH_4^+ to NO_2^- via NH_2OH (Bock et al. 1989). Although NH_2OH is unstable, it is known to accumulate in aqueous environments (Verstraete and Alexander 1973; Baxter 1979), which may cause an inhibition of algal growth in the photobioreactor. For example an *Arthrobacter* sp. is able to produce NH_2OH in significant amounts that were shown to inhibit the growth of *Chlorella vulgaris* (Berger et al. 1979).

No NO_3^- formation was detected, probably because of the inhibition of NO_2^--oxidizing bacteria by NH_4^+ NH_3 or NO_2^- (Anthonisen et al. 1976; Suthersan and Ganczarczyk 1986). Another explanation for the absence of nitrate is the denitrification of NO_2^- without the formation of any intermediate NO_3^-. In the literature, bacteria are described as being able to oxidize NH_4^+ and reduce NO_2^- to gaseous compounds (Robertson et al. 1989, 1995; Bock et al. 1992). These bacteria probably developed in the photobioreactor and were responsible for the formation of NO_2^- and its subsequent disappearence from the medium.

It was not possible to establish a steady population of algae and bacteria in the continuous cultivation, so a cultivation of bacteria without algae to fix NH_4^+ in biomass and to reduce the TOC of liquid manure seems to be the most promising alternative.

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