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SCIENCE DIRECT®
Bioresource Technology 96 (2005) 55–58
BIORESOURCE TECHNOLOGY

Biotreatment of wastewater using aquatic invertebrates, *Daphnia magna* and *Paramecium caudatum*

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Received 7 April 2003; accepted 27 January 2004
Available online 23 August 2004

Abstract

A number of major changes have occurred over the past few years, which give cause for a re-examination of conventional wastewater treatment methods. Among these are growing problems of worldwide energy and food shortages and nutrients not removed by conventional secondary processes causing algal blooms and other problems in the receiving waters. The global increase in wastewater calls for innovative low cost technology approaches to its recycling. Biotreatment systems, utilizing living organisms are receiving growing attention since they are ecologically sound, cheap and applicable in areas without land constraints. Filter feeders (both invertebrates and vertebrates) are promising in this area since they can remove suspended organic matter and bacteria, even in the size range of microns. In the present study biological treatment of municipal wastewater using two invertebrates—*Paramecium caudatum*, a protozoan and *Daphnia magna*, a cladoceran was investigated. Analysis at pre-experimental and post-experimental stages revealed the potential of these species in abatement of water pollution. *D. magna* was more efficient than *P. caudatum* in laboratory-scale studies.

Keywords: Aquatic treatment systems; Biochemical oxygen demand; Coliform bacteria; *Paramecium caudatum*; *Daphnia magna*; Biological treatment

1. Introduction

With the change from an agrarian based society to an industrial one and the consequent concentration of population, the problem of wastewater has become acute. Wastewater treatment although important from public health, ecological, aesthetic and other points of view, is generally given a low priority, especially in developing countries where there are many competing demands on the limited funds for development. High costs of construction and maintenance of sewage treatment plants is a deterrent factor. Most of the untreated wastes find their way to the nearest water source.

Aquatic treatment systems are low cost technology approaches that can be used in developing countries for recycling of wastewater, although they tend to be land intensive. The harmful effects of chemical treatment also makes ecological alternatives attractive. These systems include the use of plants, invertebrate zooplankton as well as fishes in culture operations. Detritus, algae, bacteria etc. are removed by primary consumers and become available to higher trophic levels. This results in water quality improvement along with production of useful biomass, which adds cost effectiveness to the process.

The aquatic fauna considered for use in wastewater treatment include protozoans, cladocerans and a wide variety of fishes, clams, oysters and lobsters. Although abundant in wastewaters, the contribution of protozoans to wastewater treatment was considered negligible until recently. Protozoa in pure culture flocculate particulate matter and bacteria, which aids in both clarification of the effluent and formation of sludge (Curds, 1963). Absence of protozoa in full scale plants results in turbid and inferior quality effluent. Bacterial removal has been attributed to the predatory activity of protozoa (Curds and Cockburn, 1970).

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0960-8524/$ - see front matter
doi:10.1016/j.biortech.2004.01.008

Previous studies have shown that in *Paramecium caudatum* a soluble polysaccharide changes the surface charge of the suspended colloidal particles present (Curds, 1963). Protozoans are known to feed on pathogenic bacteria including those causing diphtheria, typhus, cholera as well as fecal bacteria like *Escherichia coli* (Curds and Hawkes, 1975). The continual cropping of bacterial population dissipates energy from water. *Daphnia* are zooplanktonic cladocerans, which aid in biorecycling by consumption of algae, protozoa, bacteria and suspended organic matter. A striking feature is that they can survive in waters with low dissolved oxygen (Dinges, 1982). Loedolff (1964) evaluated the role of cladocera in stabilization pond system in South Africa and reported a significant reduction in turbidity at times when cladoceran populations were high. Frook (1974) conducted tests on *Daphnia pulex* culture on the removal of suspended solids from effluents of activated sludge treatment plants. Gellis and Clarke (1935) working on cladocera found that the organisms needed particulate food and could possibly utilize colloidal particles and bacteria.

The present study was carried out to determine the efficiency of the two invertebrates in improving the water quality of municipal wastewater. The removal of two important parameters—biochemical oxygen demand and coliforms were given emphasis.

2. Methods

2.1. Collection and culture of test organisms

Paramecium caudatum and *Daphnia magna* were selected for laboratory-scale studies. They were collected from the local ponds. *Paramecium* was cultured by the hay infusion method for ciliates. One litre of pond water or tap water was boiled and a handful of hay (size 1 in. length and 8–10 pieces in 100 ml of water) was added to it. This was allowed to boil for an additional 10 min. The mixture was cooled and allowed to stand for 2 days before inoculating it with the *Paramecium*.

Daphnia was reared in a simple culture medium, the manure-soil medium developed by Banta and modified by Anderson (1944), by supplementing it periodically with yeast. The medium was made by mixing 5 g dried sheep manure, 25 g garden soil or sandy mud, and 1 l pond or tap water. This mixture was allowed to stand for 2 days at room temperature, then strained through bolting cloth with mesh openings of approximately 0.15 mm. During straining, some of the finer soil particles also passed through the cloth. The filtrate was set aside for a week or more and the solid residue was discarded. To make the final medium, one part filtrate was mixed with 6–8 parts pond or dechlorinated tap water. The original filtrate may be kept indefinitely before the final medium is made. For individual rearing, 100 ml medium was dispensed into 125 ml wide mouth glass vessels and inoculated with one *Daphnia* per bottle.

The test organisms were acclimated to laboratory conditions before commencing the treatment.

2.2. Municipal wastewater

Municipal wastewater collected from the open sewers of Kozhikode city, Kerala was used for the experiments. Grab samples were collected from four different locations and then mixed to give an integrated sample. This sample was then analyzed for various physicochemical and bacteriological parameters including pH, electrical conductivity, total hardness, calcium, magnesium, nitrate nitrogen, phosphate phosphorous, sulphate, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand and total coliforms as APHA (1995). Table 1 shows the characteristics of the sewage used for experiments.

2.3. Experiments

Aliquots of integrated sewage were diluted with dechlorinated tap water to give experimental sewage for the experiments. 5, 10, 15 and 20 ml sewage per litre of tap water was used for this. The experiments were carried out in five litre capacity glass troughs. Laboratory-scale experiments were conducted in ten replicates using *D. magna* and *P. caudatum* for treatment periods of 15 and 7 days respectively. *Paramecium* was introduced for a treatment period of 7 days taking into account its rapid multiplication rate. *Daphnia*, with a slower multiplication rate was kept for a treatment period of 15 days. In the experiments using *P. caudatum*, the protozoan was introduced at 20 organisms per ml in each of the four sewage dilutions (5, 10, 15 and 20 ml/l). Neonates of *D. magna* were selected for experiments and

Table 1
Physicochemical characteristics of undiluted sewage water

No.	Parameter	Value
1.	Colour	Gray
2.	Temperature (°C)	27
3.	Odour	Foul
4.	pH	7.85
5.	Electrical conductivity (μS)	315
6.	Total hardness (mg/l)	236
7.	Calcium (mg/l)	65
8.	Magnesium (mg/l)	18
9.	Nitrate nitrogen (mg/l)	2.41
10.	Phosphate (mg/l)	2.08
11.	Sulphate (mg/l)	16
12.	Suspended solids (mg/l)	240
13.	Dissolved oxygen (mg/l)	1.2
14.	BOD_5, 20 °C	300
15.	COD (mg/l)	390
16.	MPN/100 ml	43×10^4