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Molecular characterization of bacterial communities in algal–bacterial photobioreactors treating piggery wastewaters

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ABSTRACT

The influence of the loading rate and the seasonal environmental conditions on the bacterial communities established in High Rate Algal Ponds (HRAPs) treating diluted swine manure was investigated using 16S rRNA Denaturing Gradient Gel Electrophoresis (DGGE) and further phylogenetic analysis. Two HRAPs were successfully operated at different loading rates (10 and 20 times diluted swine manure, resulting in average Surface Organic Loading Rates (SOLR) ranging from 13 to 99 $g\,m^{-2}\,d^{-1}$ and from 21 to 180 $g\,m^{-2}\,d^{-1}$, respectively) for 9 months. The DGGE analysis revealed a high diversity of bacteria symbiotically living with microalgae. The environmental conditions rather than the organic loading rate were the most significant factors determining the bacterial community structure. Hence, despite a higher biodiversity was recorded in summer, both HRAPs presented a similar community regardless of their different organic loading. Most of the species (≈54%) belonged to *Verrucomicrobium*, an ubiquitous phylum of aerobic bacteria commonly found in eutrophic environments. *Firmicutes* and *Proteobacteria* (*Gammaproteobacteria*) were the other dominant phyla. Some of the bacteria present in the HRAPs were highly resistant to UV radiation and floc-forming species, which might explain the reasonable good settling characteristics of the algal–bacterial biomass in the ponds. The present work provides new insights on the complex interactions between microalgae and bacteria in algal-based wastewater treatment systems.
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1. Introduction

Low-cost wastewater treatment systems have received an increasing attention in the last years, especially in the livestock farming context, where large quantities of highly polluted wastewater are generated and the cost of conventional treatments might be prohibitive.

Microalgae-based systems, a technology originally pioneered by Oswald and co-workers in the early 1950s, are one of the most promising low-cost systems (Grönlund et al., 2004; Oswald and Gotaas, 1997). Microalgae-based systems allow for a simultaneous organic matter and nutrients removal at minimal energy costs (solar-powered photobioreactors) based on a mutually beneficial relationship established between microalgae and bacteria during wastewater treatment. Thus, microalgae use the carbon dioxide and nutrients produced during the bacterial degradation of organic matter and release photosynthetically the oxygen required by aerobic bacteria (Muñoz and Guieysse, 2006). The algal–bacterial biomass generated in the biodegradation process can be then used as a raw material for biofertilizer or bioenergy production (Mulbry et al., 2005, 2010). Among microalgae-based systems, High-Rate Algae Ponds (HRAP) are likely the most common photobioreactor configuration employed in wastewater treatment processes. These systems are well mixed reactors with a high surface/volume ratio that provides the high solar irradiance required for microalgae growth. However, despite most engineering aspects of this technology are well understood, there is a significant gap of knowledge regarding the dynamics of microbial populations driving the process.

In this context, most of the efforts have been focused on the study of microalgae population dynamics and few studies have been devoted to the identification of bacterial populations (García et al., 2000). The few studies available were carried out some decades ago using conventional microbiological techniques, which today are known to provide a limited insight of the biodiversity present in the process (Allen and Garrett, 1977; Sanz and Köchling,

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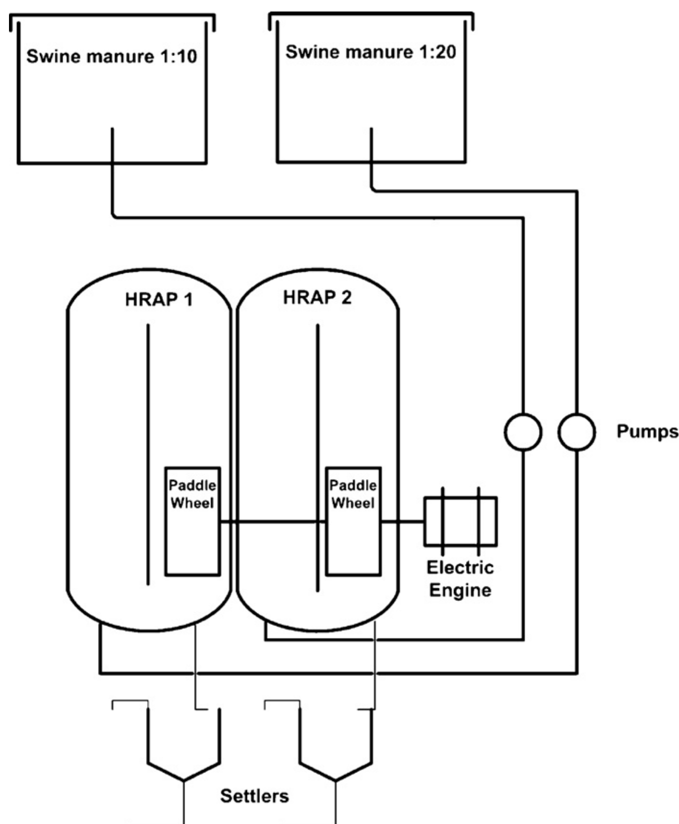

Fig. 1. Schematic illustration of the pilot plant.

2007). It is widely accepted that conventional microbiological techniques such as isolation of pure cultures or metabolic, biochemical, and morphological assays only allow for a partial identification of the bacterial community since the number of cultivable bacteria ranges from 5 to 15% of the total number of bacteria (Amann et al., 1995; Amann and Wagner, 1998). Molecular biology techniques have emerged as the cornerstone of microbial ecology research in the last decade. These techniques have resulted in new insights in microbiology that have helped improving the design and operation of conventional biological processes (Faulwetter et al., 2009; Janssen et al., 2001; Sanz and Köchling, 2007; Van Lier et al., 2001). Of them, denaturing gradient gel electrophoresis (DGGE), a technique based on the polymerase chain reaction (PCR) and amplification of 16S rDNA sequences, can provide a rapid and overall picture of the bacterial community structure (Muyzer, 1999).

The aim of the present work was to determine the bacterial community structure and dynamics in HRAPs treating diluted swine manure at different loading rates under different seasonal environmental conditions. For this purpose, samples were periodically drawn from outdoor HRAPs and subjected to DGGE analysis.

2. Materials and methods

2.1. Reactor operation

The HRAPs were located in a full-scale swine manure treatment plant placed in Hornillos de Eresma (Valladolid, Spain). Both HRAPs had the same dimensions (2.3 m long, 0.70 m wide, 0.30 m deep and a total volume of 464 L) (Fig. 1). The ponds were continuously mixed by a six-blade paddle wheel and fed with 20 and 10 folds diluted swine manure (namely HRAP 1 and HRAP 2, respectively) at a hydraulic residence time of 10 days. Manure was obtained from the primary treatment of the facility (screening and sedimentation). The effluent of the ponds was clarified in static settlers working at superficial hydraulic load of 2.3 m^{-3} m^{-2} d^{-1}. The settlers were purged twice a week.

The average COD (Chemical Oxygen Demand) concentrations of the inlet wastewater in HRAP 1 during winter, spring and summer were 506 ± 182, 679 ± 201 and 1716 ± 629 mg L^{-1}, while in HRAP 2 these average concentrations were 1009 ± 182, 1195 ± 767, 3338 ± 1651 mg L^{-1}. These COD concentrations in the wastewaters resulted in average SOLR from 13 to 99 g m^{-2} d^{-1} and from 21 to 180 g m^{-2} d^{-1}, respectively. The SOLRs were not constant throughout the experimentation time and the oxygenation capacity of the ponds was overcome during summer. This increase in SOLR, together with the increase in temperature and solar irradiance, significantly hindered the discussion of the results. Despite the increasing SOLRs, the HRAPs showed a good performance in terms of organic matter elimination and NH_4^+ oxidation under the different conditions. In addition, it must be stressed that variable concentrations in swine manure is the most common scenario in real livestock treatment plants. Average TKN (Total Kjeldahl Nitrogen) concentrations of 65 ± 21, 61 ± 28 and 156 ± 44 mg L^{-1} were recorded in the inlet wastewater of pond 1 during winter, spring and summer, respectively, while in pond 2 the TKN concentrations were 126 ± 32, 103 ± 59 and 314 ± 108 mg L^{-1}, respectively, for the same periods. A more detailed description of the experimental set-up, operational conditions, wastewater treatment performance, microalgae populations and protocols can be found in de Godos et al. (2009).

2.2. Collection of samples

Samples were monthly collected from the cultivation broth of both HRAPs from January 2008 till September 2008 (9 samples per reactor). Samples were placed in sterile polypropylene tubes and stored at −20 °C.

2.3. DNA extraction

DNA was extracted from the HRAP samples using the FastDNA® Spin kit for Soil (MPBiomedicals, LLC). The quality of the extracted DNA was examined on a 1.2% (w/v) agarose gel. DNA was stored at −20 °C.

2.4. PCR and DGGE analysis

The primers 0968F-GC (5′-CGCCCGGGGCGCGCCCCGGGCGGGG-CGGGGGCA CGGGGAACGCGAAGAACCTTAC-3′) and 1401R (5′-CGGTGTGACAAGACCC-3′) were used to amplify the V6-V8 region of eubacterial 16S ribosomal DNA (rDNA) (Nübel et al., 1996).

PCR was performed using the *Taq*-& GO™ Mastermix (MPBiomedicals, Europe). PCR mixtures (50 μl) contained 10 μl of *Taq*-& GO™ Mastermix, 1 μl of each primer 0968F-GC and 1401R, 3 μl of diluted DNA and 35 μl of sterile distilled water. Amplification of 16S rDNA were carried out using a thermocycler (iCycler™ Bio-Rad, US) with the following program: 5 min of pre-denaturation at 94 °C, 32 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 sand elongation at 72 °C for 60 s and a final elongation at 72 °C for 10 min. Aliquots of 4 μl were used to analyse the size and yield of PCR products on a 1.8% (w/v) agarose gel. In each amplification set a negative control, (no DNA added), was included in parallel.

PCR products were loaded into a 10% (w/v) polyacrylamide gel with a denaturant gradient of 42–67%. The gel was subjected to electrophoresis at a constant voltage and temperature of 85 V and 60 °C for 16 h using the DCode™ Universal Mutation Detection

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