Enumeration and isolation of facultative anaerobic bacteria in an upflow anaerobic sludge blanket reactor treating wastewater from a gelatine industry

Angelica Marquetotti Salcedo Vieira¹, Rosangela Bergamasco¹, Marcelino Luiz Gimenes¹, Celso Vataru Nakamura² and Benedito Prado Dias Filho²*

¹Departamento de Engenharia Química ²Departamento de Análises Clínicas, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. *Author for correspondence. e-mail: bpdfilho@uem.br

ABSTRACT. This experiment measured levels of facultative anaerobic bacteria in an Upflow Anaerobic Sludge Blanket (UASB) reactor treating wastewater from a gelatin industry. The microorganism quantification showed similar values in granular sludge bed and fluidized zone. In the settling compartment of reactor, the bacteria were two logs lower. These communities are composed mainly of Gram-negative rods. The most abundant genera isolated were Acinetobacter, Aeromonas, Citrobacter, Escherichia, Klebsiella and Stenotrophomonas. The significance of the bacteria isolated from UASB reactor for public health is unknown. Some species are permanent residents; other are found only in a fraction of the population, and still other species are opportunistic pathogens that cause human infections. In the present study, none of the predominant bacteria belonged to the group of strict pathogens.

Key words: UASB-reactor, wastewater, gelatin, facultative anaerobic bacteria, Gram-negative rods.

RESUMO. Enumeração e isolamento de bactérias anaeróbias facultativas num reator de fluxo ascendente e manta de lodo tratando efluente de uma indústria de gelatina. Neste trabalho foram determinados os níveis de bactérias anaeróbias facultativas em Reator de Fluxo Ascendente e Manta de Lodo (UASB) utilizado no tratamento de efluente de indústria de gelatina. A quantificação dos microrganismos apresentou similar valor na manta de lodo granular e na zona de fluidização. No compartimento de sedimentação do reator as bactérias foram dois logs menores. Estas comunidades são compostas principalmente de bacilos Gram-negativos. Os mais abundantes gêneros foram Acinetobacter, Aeromonas, Citrobacter, Escherichia, Klebsiella e Stenotrophomonas. O significado para a saúde pública das bactérias isoladas no reator UASB é desconhecido. Algumas espécies são residentes permanentes, outras são encontradas em somente uma parte da população, e ainda outras espécies são patógenos oportunistas que causam infecção humana. No presente estudo, nenhuma das bactérias predominantes pertence ao grupo dos patógenos estritos.

Palavras-chave: reator UASB, efluente industria de gelatina, bactérias anaeróbias facultativas, bacilos Gram-negativos.

Introduction

The upflow anaerobic sludge blanket reactor has become the most popular high-rate reactor for anaerobic biological treatment of industrial and city wastewater. The reactor is typically divided into four compartments: the granular sludge bed, the fluidized zone, the gas-solids separator, and the settling compartment. The granular sludge bed is located in the bottom of the reactor. The wastewater is pumped into the bottom of the reactor and passes upward through the granular sludge bed. Here the organic compounds are biologically degraded and biogas is produced. In the top, just above the granular sludge bed, a fluidized zone is developed, due to biogas production. In this zone, further biological degradation may take place. The biogas is separated from the liquid in the gas-solids separator. Granules with good settling abilities settle back through the fluidized zone to the granular sludge bed. In UASB-type reactors, the biomass retention is promoted by bacterial self-aggregation into dense granules. The aggregation enhances the performance of high-rate anaerobic reactors, since
the good settling of granules minimizes the biomass washout and the close cell packing optimizes the interspecies metabolites exchange. (Schmidt and Ahring, 1996).

The end of the treatment sequence involves disposal of the remaining stabilized effluent. Effluent disposal poses a serious, world-wide problem. Because of the increased environmental awareness and the stringent environmental standards governing the disposal set by different environmental protection agencies, the utilization of effluent in agricultural production has been gaining interest and attention in recent years. It offers economic and nutrient recycling advantages over the traditional disposal options, such as incineration for dry sewage and sea disposal (Stone et al., 1998). Nevertheless, potential risks from accumulation of heavy metals and organic compounds, as well as pathogen contamination, must be considered.

The present study aims to (i) determine the predominant facultative bacteria found in the UASB, (ii) to determine the proportion of each predominant type in the total population, and (iii) to determine whether there were potential pathogens among these predominant organisms.

**Material and methods**

The facultative anaerobic population measurements carried out in this study is from an upflow anaerobic sludge blanket reactor, used to treat wastewater from a gelatin industry. The reactor throughput was 46 m$^3$ h$^{-1}$ for a retention time of 22.6 h. Characterization of influent and effluent were performed according to Standard Methods (Apha, 1995).

The suspended solid or sludge (1mL) from different compartments of the Upflow Anaerobic Sludge Blanket (UASB) was diluted with phosphate-buffered saline to 10mL into 20-mL containing 15 to 20 glass beads of 0.5mm. The serum bottle was placed on a Vortex mixer for 2 min and the bacterial flocs were disrupted. A similar treatment was applied to the fourth successive decimal dilution tube. Samples of 0.1mL of either homogenized or dilutions of these in PBS were spread on Nutrient agar and MacConkey agar plates for colony plate counts, for total culturable and Gram-negative bacteria, respectively. The influent supernatant (IM) clarified by centrifugation was also used in the experiments. Duplicate plates were prepared for each sample. All plates were incubated at 37°C for 24-48h. Following incubation, colony counts were determined, and representative colonies subcultured for identification. Mean bacteria number was calculated as the average of each duplicate set and expressed as colony forming units (CFU) mL$^{-1}$ of sludge. Cellular morphologies were determined by brightfield microscopy of Gram-stained preparations. The microorganisms were identified according to standard methodologies, using biochemical tests (Holt et al. 1994) as well as standard techniques (MacFaddin, 1980; Murray et al., 1995) used in our laboratory, and BBL Crystal Identification Systems (BBL Microbiology Systems, Cockeysville, Md).

**Results and discussion**

The UASB reactor used to treat the gelatin industry wastewater has a working volume of 46 m$^3$/h at retention time of 22.6 h, with approximately 290 Nm$^3$/h biogas production, including 22000 ppm of H$_2$S. Chemical oxygen demand (COD) presents an average reduction of 83%, as well as the suspended solids, with reductions of 82% for Volatile Suspended Solids (VSS), 61% for Fixed Suspended Solids (FSS) and 79% for Total Suspended Solids (TSS). The analyses of Total Phosphates, Oils and Greases demonstrate a reduction of 34% and 76% respectively, after the biological treatment. After treatment in the UASB reactor the sulfide concentration increased in 257%, which is a significant increase (Table 1). The average of the reactor fluid pH was 7.8, with a normal range from 7.4 to 8.0.

**Table 1.** Characterization of the influent and effluent of USAB reactor from which granules were obtained for analysis.

| **Concentration**       | Influent | Effluent |
|-------------------------|----------|----------|
| Chemical Oxygen Demand  | 6678     | 1333     |
| Volatile Suspended Solids | 1629    | 279      |
| Fixed Suspended Solids  | 346      | 134      |
| Total Suspended Solids  | 1975     | 412      |
| Total Phosphate         | 5.37     | 3.54     |
| Oil and Grease          | 1388     | 326      |
| Sulfide                 | 71       | 253      |

*All values are in mg/l.*

Preliminary studies were performed on the sludge samples in order to determine the most suitable medium for counting the bacteria along the suspended solid or sludge. Our attention was focused on enumerating cultivable microbial populations, and in all cases the highest numbers were obtained on the nutrient agar (data not shown). Facultative anaerobic bacteria were determined in four separate samples, taken during a 12-month period. The number of cultivable cells varied from
Facultative anaerobic bacteria in a upflow anaerobic reactor 259

The levels of facultative anaerobic bacteria in the different compartments of the Upflow Anaerobic Sludge Blanket (UASB) reactor treating wastewater from a gelatin industry are presented in Figure 1. The quantification of microorganism showed similar values in the granular sludge bed and fluidized zone. Microorganism isolates from UASB samples contained low proportion of Gram-positive, when compared to Gram-negative. These communities are composed mainly of Gram-negative rods. The physiological types of bacterial flora in the three compartments and in the effluent varied minimally (data not shown).

Table 2. Gram-negative isolates in sludge from UASB reactor

| Group                              | Identification           | No. of isolates from: |
|------------------------------------|--------------------------|-----------------------|
|                                    |                          | Granular sludge bed   | Fluidized zone | Settling compartment |
| Facultatively anaerobic            | *Klebsiella oxytoca*     | 23                    | 32            | 6                    |
| Gram-negative rods                 | *Citrobacter freundii*   | 6                     | 8             | 1                    |
|                                    | *Aeromonas veronii*      | 6                     | 8             | 1                    |
|                                    | *Aeromonas hydrophila*   | 9                     | 13            | 2                    |
|                                    | *Escherichia fergusonii* | 1                     | 1             | 0                    |
|                                    | *Escherichia coli*       | 3                     | 4             | 0                    |
| Gram-negative                       | *Acinetobacter iwoffii*  | 23                    | 35            | 6                    |
| aerobic/microaerophilic rods       | *Stenotrophomonas maltophilia* | 3            | 4             | 0                    |
Acknowledgements

This research was supported by the Post-graduation program from the Departamento de Engenharia Química, Universidade Estadual de Maringá and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

APHA - AMERICAN PUBLIC HEALTH ASSOCIATION. Standard methods for examination of water and wastewater. 19. ed., Washington: APHS, 1995.

DENTON, M.; KERR, K.G. Microbiological and clinical aspects of infection associated with Stenotrophomonas malthophilia. Clin. Microbiol. Rev., Washington, DC, v. 11, p. 57-80, 1998.

HIRD, D.W. et al. Enterobacteriaceae and Aeromonas hydrophila in Minnesota Frogs and Tadpoles (Rana pipiens). Appl. Environ. Microbiol., Washington, DC, v. 46, p. 1423-1426, 1983.

HOLT, J. G et al. Bergey’s manual of determinative bacteriology. 9. ed. Baltimore: The Williams & Wilkins Co, 1994.

KROVACEK, K. et al. Comparison of putative virulence factors in Aeromonas hydrophila strains isolated from the marine environment and human diarrheal cases in southern Italy. Appl. Environ. Microbiol., Washington, DC, v. 60, p. 1379-1382, 1994.

MAC FADDIN, J.F. Biochemical tests for identification of medical bacteria. Baltimore: The Williams & Wilkins Co., 1980.

MURRAY, P.R. et al. Manual clinical microbiology. 6. ed. Washington: American Society for Microbiology, 1995.

PATHAK, S.P. et al. Seasonal distribution of Aeromonas spp in river water and isolation from river fish. J. Appl. Bacteriol., Oxford, v. 65, p. 347-351, 1988.

SCHMIDT, J.E.; AHRING, B.K. Granular sludge formation in upflow anaerobic sludge blanket (UASB) reactors. Biotechnol. Bioeng., New York, v. 49, p. 229-246, 1996.

STONE, R.J. et al. Engineering properties of sewage sludge in Trinidad. J. Agric. Eng. Res., London, v. 70, p.21-230, 1998.

Received on September 09, 2002.

Accepted on October 13, 2003.