

EVALUATION OF THE EFFICACY OF MATERIALS AS ANAEROBIC WASTEWATERS TREATMENT SUPPORTS BY PHOSPHOLIPID ANALYSIS

Arnáiz, M. C.; Ruiz, C.; Gómez, E.; García, I.; Escot, E. Aguilar, E.; Medialdea, J. M. and Lebrato, J.*

Grupo TAR. Department of Chemical and Environmental Engineering. Technical College. University of Seville. C/ Virgen de África, 7. 41011-Seville. Spain.

* Author to whom all correspondence should be addressed. Fax: 34 5 4282777. e-mail: lebrato@cica.es

Introduction

Anaerobic digestion is a suitable process for the treatment of wastewaters containing a high concentration of organic carbon (*e.g.*, food processing wastes, pig farm wastes, wine and brewery industries wastes).

The advantages of anaerobic wastewater treatment processes, in comparison with aerobic treatment processes, are well known (Bull *et al.*, 1982). The disadvantage of lower degradation rates has been considerably improved by new reactor designs that maintain high concentration of microorganisms (Lettinga *et al.*, 1980; Lettinga *et al.*, 1997). One of the possibilities to obtain high levels of biomass is to provide support materials into the reactor (Henze and Harremoes, 1982; Lebrato *et al.*, 1993). Many materials have already been tested (Huysman, *et al.*, 1983; Maqueda *et al.*, 1995). However, a material is usually selected on the basis of substrate removal rate or biogas flow rate, not on the underlying biofilm features.

An important parameter to be measured is anaerobic fixed biomass. Many methods are described for quantifying biofilms (Lazarova and Manem, 1995), but actually the most widespread are gross measures such as volatile solids (Anderson *et al.*, 1994; Hawkes *et al.*, 1995), plate count of colony forming unity (Fukui and Takii, 1990) and most probable number (Anderson *et al.*, 1994), if any is measured. Disadvantages of these gross methods of biomass estimation are detailed in Lazarova and Manem (1995) and are mainly focused on time-consumption, low repeatability, over or underestimation. More refined methods can be found in Ehlinger *et al.* (1987) that determine the contribution of exopolysaccharides to biofilm formation in an anaerobic filter, and Kuba *et al.* (1990) that estimates protein concentration in an anaerobic fluidized reactor.

Phospholipids, a cell wall component, offer many advantages over other assays for selective cellular biomass estimation in biofilms, and their determination by colorimetric methods is relatively simple, reproducible and sensitive (Lazarova and Manem, 1995). Lipids have been

widely used in environmental samples (White *et al.* 1979; Balkwill *et al.*, 1988; Findlay *et al.*, 1989) and recently applied to aerobic wastewater biofilm (Zhang and Bishop, 1994).

In this work, we assess the efficacy of phospholipid analysis in determining the effectiveness of materials as anaerobic wastewater treatment supports.

Materials and methods

The experiments were carried out in batch reactors designed by Lebrato (1990) that allow to work under anaerobiosis and take samples when required. The reactors consisted of screw-cap bottles (125 mL of volume) with a one-way valve for gases exit, containing 10 mL of material support, 10 mL of inoculum and 80 mL of synthetic feed. The support materials tested were perlite (supplied by Europerlita S. A., Spain) and sepiolite (Sepitol 30/60 supplied by Tolsa S.A., Spain). The inocula were obtained from an anaerobic continuously stirred tank reactor that processed a mixture of 1.0 g/L for potassium acetate and 0.5 g/L for sodium lactate. The synthetic mineral medium is a modification of that by Shelton and Tiedje (1984) with a mixture 2:1 for potassium acetate and sodium lactate respectively as the carbon source, at a final concentration of 600, 800 and 1000 mg/L of TOC. Blank support-free reactors were also prepared. The reactors were kept at a temperature of 35 °C and a stirring speed of 250 rpm. The experiments were repeated three times for each carbon concentration.

The procedure followed for the phospholipid analysis was a modification of that by Findlay *et al.* (1989). It consists of: a) a sample of biomass-laden particles (0.5-1.5 mL) was withdrawn from the reactor and gently washed with 100 mL of distilled water to remove any unattached biomass; b) this sample was then transferred to a 100 mL screw-cap bottle and 20 mL of chloroform, 20 mL of methanol and 20 mL of distilled water were added. The extraction mixture was shaken for 1 hour; c) 45 mL of the aqueous upper phase was aspirated from the bottles and subsamples of 5 mL of chloroform were transferred to COD test tubes; d) after chloroform removal under a nitrogen stream, 2.7 mL of the potassium persulfate solution was added and test tubes were heated in an oven at 105 °C for 1 hour; e) the phosphate released by digestion was determined by adding 0.6 mL of the ammonium molybdate solution and 2.7 mL of the malachite green solution; f) the absorbance at 610 nm was then read using a spectrophotometer. The concentrations of phosphate were calculated by using the regression line from a standard curve obtained by digesting 2.5, 5, 10, 20, 40, 60, 80, 100 and 150 μ L of a 1 mM glycerol phosphate solution.

Attached solids were measured following Livingston and Chase (1991): a sample of biomass-laden particles (0.5-1.5 mL) was withdrawn from the reactor and gently washed with 100 mL of distilled water to remove any unattached biomass. This sample was then transferred to a weighed crucible and placed in an oven at 105 °C for 24 hours to remove all unbound moisture.

The crucible was reweighed and placed in a furnace at 560 °C for 1 hour to burn off all the biomass present, followed by a further reweighing. Because perlite and sepiolite themselves showed some ignition loss (2 % and 4 % respectively), the obtained values were corrected accordingly. The biomass loading was calculated as the difference between the two weighings divided by the total volume of support material present in the sample.

Biodegradation was measured by TOC removal. Samples for phospholipid analysis and attached solids were taken when the percentage of TOC removal was around 90 % at the day 12 after starting-up the reactors.

Results and discussions

Obtained data are shown in Table 1 for blank reactors, Table 2 for perlite reactors and Table 3 for sepiolite reactors.

TOCinitial (mg/L)	nmol Pi/mL Mean (STD)	Total solids (mg/mL)	$\mu\text{g TS/nmol Pi}$
600	49.04 (6.32)	1.14	23.25
800	32.67 (0.87)	1.08	33.06
1000	29.90 (1.19)	0.94	31.44

Table 1. Biomass loading expressed as lipid phosphate concentration and mass of dry biomass per unit volume of reactor. Last column shows conversion factors from lipid phosphate concentration to living cell weight. The average of the conversion factors is 29.25 with the STD of 5.26 ($\mu\text{g TS/nmol Pi}$).

TOCinitial (mg/L)	nmol Pi/mL Mean (STD)	Total solids (mg/mL)	$\mu\text{g TS/nmol Pi}$
600	160.65 (3.67)	8.3	51.67
800	132.02 (2.49)	5.3	40.15
1000	176.96 (4.58)	8.6	48.60

Table 2. Biomass loading expressed as lipid phosphate concentration and mass of dry biomass per unit volume of perlite support. Last column shows conversion factors from lipid phosphate concentration to living cell weight. The average of the conversion factors is 46.81 with the STD of 5.97 ($\mu\text{g TS/nmol Pi}$).

TOCinitial (mg/L)	nmol Pi/mL Mean (STD)	Total solids (mg/mL)	$\mu\text{g TS/nmol Pi}$
800	313.82 (24.71)	16.7	53.21
1000	319.82 (5.74)	15.6	48.78

Table 3. Biomass loading expressed as lipid phosphate concentration and mass of dry biomass per unit volume of sepiolite support. Last column shows conversion factors from lipid phosphate concentration to living cell weight. The average of the conversion factors is 51.00 with the STD of 3.13 ($\mu\text{g TS/nmol Pi}$).

The main objective in measuring lipid phosphate concentrations in the biofilm was to find the living cell biomass. A comparison of the conversion factors between perlite and sepiolite samples shows a mean of 48.48 $\mu\text{g TS/nmol Pi}$ with the STD of 5.05 $\mu\text{g TS/nmol Pi}$, and the coefficient of variation of 10 %. This average factor is approximately ten times higher than those of Findlay *et al.* (1989) and Zhang and Bishop (1994), 4.785 $\mu\text{g-cell/nmol}$ and 4.81 $\mu\text{g TS/nmol}$, respectively. It should be mentioned that in both works conversion factors were obtained by enrichment cultures of free-living cells, so extracellular polymeric substances (EPS) could appear at lower levels. Something similar is reported by Cao and Alaerts (1995) for the specific oxygen biomass of aerobic biofilm that is almost two times higher in the suspended biomass than those of biofilm. The EPS accumulation was assumed by the authors to be the main reason. Lazarova and Manem (1995) report the limitations of selective enrichment. More data in this sense are needed for general conclusions.

A comparison between biomass loading (expressed as lipid phosphate concentration per unit volume of support) shows that sepiolite carried by almost two times the biomass that perlite did. In both cases the biomass attached per unit volume of support is higher than the biomass per unit volume of reactor in the blank experiments. Therefore, phospholipid analysis of attached biomass in batch reactor seems to be a good method for selecting material supports, based on biomass loading.

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