Technical note

Preliminary observations on polyhydroxybutyrate metabolism in the activated sludge process

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Abstract

The synthesis and degradation of polyhydroxybutyrate in various zones of three activated sludge plants was monitored. Good phosphorus removal appeared to coincide with anaerobic sythesis of polyhydroxybutyrate. Sufficient substrate addition was essential for synthesis of polyhydroxybutyrate and subsequent phosphorus uptake.

Introduction

Polyhydroxybutyrate (PHB) is a reservoir of carbon and energy which accumulates in a variety of micro-organisms, generally under conditions of nutrient imbalance (Dawes and Senior, 1973).

The accumulation of PHB by an *Acinetobacter calcoaceticus* var. *lwoffi* isolated from an activated sludge plant, was first demonstrated by Fuhs and Chen in 1975. Subsequent investigations indicated that a number of *Acinetobacter* strains were capable of carbon storage by this route (Lawson and Tonhazy, 1980; Lötter *et al.*, 1986a).

The metabolism of PHB by *Acinetobacter* spp. in the activated sludge process has been described in detail by Wentzel *et al.* (1986).

Microscopic examination of activated sludge for the presence of PHB has revealed an increase in the anaerobic zone and subsequent depletion on aeration (Hart and Melmed, 1982; Lötter *et al.*, 1986b). While the metabolism of PHB has not been studied in the presence of nitrate, the deleterious effect of nitrate on anaerobic zone behaviour has been well documented (Barnard, 1976; Osborn and Nicholls, 1978).

The aim of this study was to investigate the synthesis and utilisation (degradation) of PHB in the anaerobic, anoxic and aerobic zones of three Johannesburg activated sludge plants with different configurations. The volatile fatty acid levels in the feed, as well as the nitrate levels entering the anaerobic zone, were monitored at the same time, to assess the influence of these parameters on PHB metabolism.

Description of activated sludge plants

The configurations of the plants surveyed are given in Figure 1. Goudkoppies, a five-stage Bardenpho plant, was fed settled sewage at a rate of 36 Mℓ/d from a balancing tank. Module 2 of Northern Works, a three-stage Bardenpho plant, was fed at the same rate with settled sewage and recycled primary sludge. This module also incorporated an anaerobic selector and compartmentalised anaerobic reactor. Module 3 had the same configuration as the Goudkoppies plant and was fed 14 Mℓ/d settled sewage, in the same way as Module 2.

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Material and methods

Mixed liquor samples were drawn from the anaerobic, primary anoxic and secondary aerobic zones of the three plants, and immediately analysed for PHB by the method of Braunegg *et al.* (1978).

The balance tank and final effluent composition was determined by chemical analysis. Chemical oxygen demand (COD) was determined by the standard method of the American Public

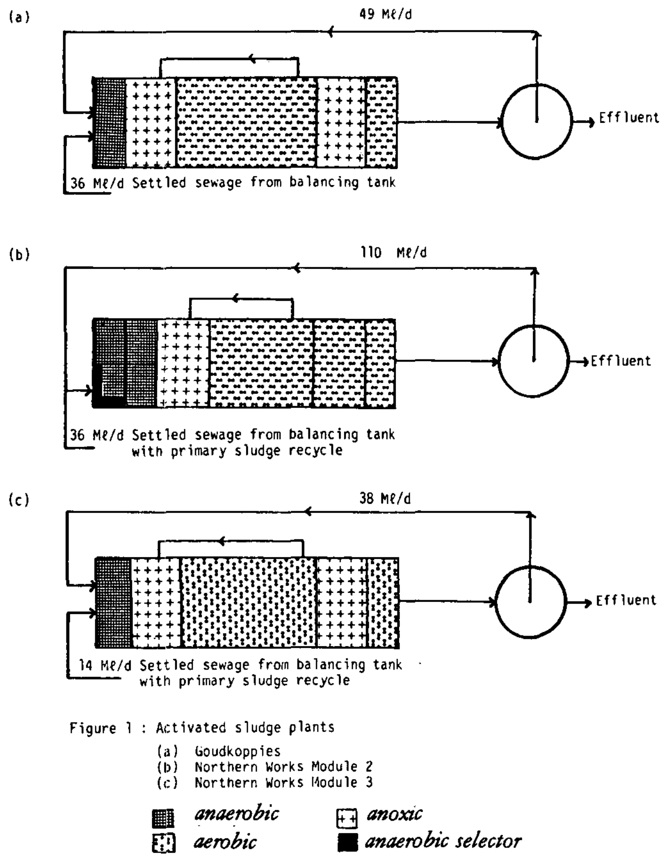

Figure 1: Activated sludge plants
(a) Goudkoppies
(b) Northern Works Module 2
(c) Northern Works Module 3

TABLE 1
AVERAGE CHEMICAL COMPOSITION OF THE INFLUENT AND FINAL EFFLUENTS DURING SAMPLING PERIOD FOR THE THREE PLANTS STUDIED

Parameter	Goud-koppies Module 2		Northern Works Module 2		Northern Works Module 3	
	Influ-ent	Efflu-ent	Influ-ent	Efflu-ent	Influ-ent	Efflu-ent
COD as oxygen	700	84	997	48	773	49
Kjeldahl nitrogen as N	49	3,0	86	2,5	63	2,6
Ammonia as N	35	0,5	32	0,6	34	0,7
Total phosphorus as P	9,3	0,6	18	1,6	16	4,6
Orthophosphate as P	6,9	0,3	11	1,4	12	4,6
Nitrate as N	–	2,7	–	4,5	–	4,8
Total volatile fatty acids as CH_3COOH	144	–	66	–	59	–

(All results expressed as mg/ℓ)

TABLE 2
RANGE OF POLYHYDROXYBUTYRATE LEVELS IN THE THREE PLANTS STUDIED

Sample description	Polyhydroxybutyrate µg/g VSS		
	Minimum value	Maximum value	Average (n = 16)
Goudkoppies Module 2			
Anaerobic zone	37	190	125
Anoxic zone	13	58	38
Primary anoxic zone	13	50	35
Secondary anoxic zone	12	40	29
Northern Works Module 2			
Anaerobic zone	15	79	47
Anoxic zone	20	49	35
Aerobic zone	12	51	28
Northern Works Module 3			
Anaerobic zone	21	55	42
Anoxic zone	20	44	32
Primary anoxic zone	4	44	25
Secondary aerobic zone	4	42	19

Health Association (1981). Orthophosphate was determined by the molybdenum blue colorimetric method (Canelli and Mitchell, 1975), and ammonia by indophenol blue reaction (Harwood and Huyser, 1970). Samples for Kjeldahl nitrogen and total phosphorus determination were digested by the method of Jirka *et al.* (1976) prior to the ammonia and orthophosphate analysis. Alkalinity was determined according to the Analytical Guide (1974).

The volatile suspended solids (VSS) content of the samples was determined by igniting at 550°C, the residue retained on a glass fibre filter and dried at 150°C (American Public Health Association, 1981). The pH of balance tank samples for volatile fatty acid determination was adjusted to 12,0 immediately after sampling.

Immediately prior to analysis, the samples were acidified and 50 mℓ samples subjected to steam distillation (Horwitz, 1980) Extracts were subjected to gas chromatographic analysis on a 10 m 530µ Carbowax 20M column with temperature programming from 80°C to 150°C.

Results and discussion

The chemical composition of the balance tank and final effluents is given in Table 1.

The relatively high volatile fatty acid content entering the Goudkoppies plant, can be attributed to the augmentation of the influent flow with that from a shallow gradient septic sewer, containing effluent from the yeast industry (Nicholls *et al.*, 1986).

The level of volatile fatty acids in the Northern Works influent was achieved by recycling approximately 50% of the raw sludge to the head of the primary clarifiers (Nicholls *et al.*, 1986).

In contrast with the consistently satisfactory performance of the Goudkoppies plant, the two modules of Northern Works did not remove phosphate to the 1,0 mg/ℓ level, as required by the effluent standard (Government Gazette, 1984).

The polyhydroxybutyrate levels, expressed as the fraction of the volatile suspended solids, in the various zones of the plants, are given in Table 2. A reduction in polyhydroxybutyrate concentration from the anaerobic zone to the zones in all three plants is evident.

While more investigations into the metabolic behaviour of polyhydroxybutyrate are required, these results indicate that efficient synthesis of poly-β-hydroxybutyrate in the anaerobic zone provides carbon for metabolism in the aerobic zone and that degradation of the polyhydroxybutyrate in this zone leads to enhanced phosphorus uptake.

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