The phenomenon of sludge pelletisation in the anaerobic treatment of a maize processing waste

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Abstract

The anaerobic treatment of certain soluble organic wastes in upflow sludge bed reactors has resulted in the formation of granular or pellet sludges which have excellent settling and thickening properties. These properties hold considerable promise for optimisation of industrial waste anaerobic treatment systems in terms of improved biomass retention and loading rates.

This paper describes digester operational conditions which resulted in the formation of sludge pellets and illustrates certain physical, chemical and microbiological characteristics of such sludge types. The possible role of feed and extracellular polymers in the mechanisms underlying the pelletisation process is discussed.

Introduction

Biomass concentration and retention are considered important factors for the successful operation of a high-rate anaerobic process when treating a soluble/colloidal type of organic waste. Various anaerobic designs are being advocated in order to achieve both a high solids concentration and subsequent effective phase separation of the digester liquor. Of particular interest and significance is the reported sludge pelletisation or granulation phenomenon which has been achieved in full-scale upflow anaerobic sludge blanket (UASB) digesters in Holland treating sugar beet and potato starch processing wastes (Lettinga *et al.*, 1979; Lettinga *et al.*, 1980; Pette *et al.*, 1980; Pette and Versprille, 1981).

Based on the excellent results achieved in the Dutch plants, it would appear that the pelletisation phenomenon holds considerable promise in terms of improved sludge settling and retention, higher organic and hydraulic load rates and costs of digester design. Rabson and Rogers (1981) in reviewing future biomass technologies, considered the study of the mechanisms of aggregate formation and operation of microconsortia pellets as a priority area for the development of new types of methane generators of the future.

A dense pellet sludge with excellent settling and thickening properties has also been developed in a full-scale upflow clarigester plant in South Africa, treating a glucose/starch waste (Ross and Smollen, 1981). This paper describes the full-scale and pilot-scale digester operational conditions which resulted in the formation of sludge pellets and illustrates certain of their physical, chemical and microbiological characteristics. A possible mechanism for the formation of such sludge types is discussed.

Anaerobic treatment of a maize processing waste

History of the full-scale clarigester plant

An industrial waste arising from the manufacture of glucose and starch from maize has been successfully treated for some 22 years in a full-scale upflow clarigester plant situated at the Bellville sewage works (Hemens *et al.*, 1962). The plant consists of three identical unheated clarigesters, each with a digester and clarifier capacity of 623 m^3 and 374 m^3, respectively (Figure 1).

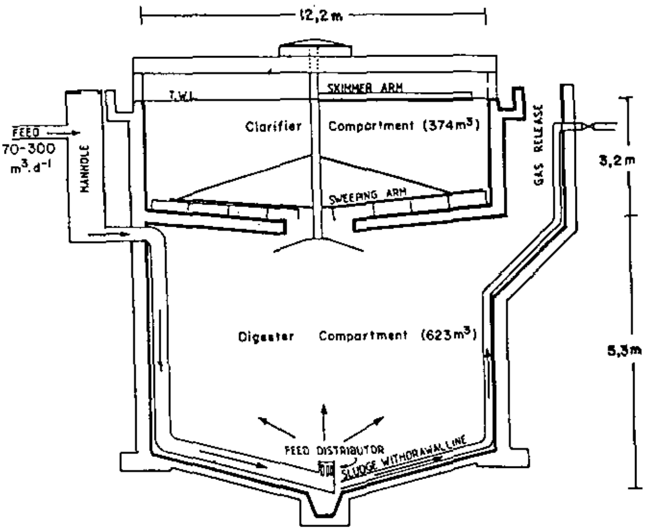


Figure 1
Full-scale upflow clarigester. Plant consists of three such units with a combined total capacity of 3000 m^3

The degree of COD reduction is 93 per cent, based on an initial mean substrate concentration of 7200 mg ℓ^{-1}. A feature of this plant was the well settling pelletised sludge that had developed over the years (Ross, 1980). This allowed suspended solids concentration of some 45 g ℓ^{-1} to be maintained in the digester compartment with a minimum loss of solids in the final effluent. In 1981/82, all the sludge had to be wasted to effect necessary repairs to the plant. This afforded an opportunity to study the pelletisation process from start-up. The clarigesters were thereafter recommissioned using primary sewage sludge as inoculum. Bacterial pellets once again formed a significant portion of the sludge at the bottom of the clarigesters some six months after acclimatisation with the glucose/starch waste had commenced.

Wastewater characteristics

The factory from which the wastewater arises processes maize (corn) for the manufacture of corn flour and a wide range of starches, dextrins, glucose syrups and by-products such as maize gluten (20-60 % protein content) and maize germ oil (54 % oil mass). The composition of the effluent discharged to the clarigester plant varies according to processing conditions but in general consists of small quantities of soluble inorganic matter, protein, amino acids and other nitrogenous substances, lactic acid, carbohydrates and also finely divided solids such as starch, gluten, fibre and powdered activated carbon (Cronje, 1973). The quality of the wastewater is such that it can be anaerobically treated without additional supply of nutrients or buffer capacity.

TABLE 1
ANALYSIS OF GLUCOSE/STARCH RAW FEED

Parameters	Mean value	Standard deviation
pH value	4,28	0,71
COD unfiltered (mg ℓ^{-1})	7 194	3 531
COD filtered (mg ℓ^{-1})	5 842	3 247
Total solids (mg ℓ^{-1})	6 041	3 003
Dissolved solids (mg ℓ^{-1})	5 020	2 901
Suspended solids (mg ℓ^{-1})	1 105	1 139
Conductivity (μS/cm)	1 475	505

An analysis of the raw feed is presented in Table 1 which represents mean values of weekly samples over a five year period. Important characteristics of this type of wastewater which are relevant to the pelletisation process are discussed below:

- About 80 per cent of the total chemical oxygen demand (COD) of the raw waste is soluble. However, some 50 per cent of the soluble COD cannot be identified by conventional liquid chromatography and the identification of these organics is the subject of a special study. The ratio of COD to total organic carbon indicates that there is no inorganic oxidisable COD. In comparison with the treatment of beet sugar wastes in Holland, volatile fatty acids constitute a very low percentage of the COD in this instance. (Pette *et al.*, 1980).

- The presence of heterogenous polymeric material in the soluble glucose/starch raw feed was verified by precipitation with 2 volumes ethanol per 1 volume feed, according to the method of Forster (1971). Chemical analyses of the precipitated polymer revealed that the organic fraction (52 %) was mainly composed of a carbohydrate and a protein (Table 2). The precipitation of an inorganic fraction (48 %) with ethanol is made possible by either the association of ions with high molecular mass polymer or by the lowering of the respective solubility products in a solvent other than water.

The molecular mass of the feed polymer was found to be as high as two million. This was determined by gel permeation chromatography using blue dextran as standard. The possible role that this polymer plays in the pelletisation process is currently being investigated.

TABLE 2
ANALYSIS OF POLYMER EXTRACTED FROM SOLUBLE GLUCOSE/STARCH FEED

Parameter	Percentage concentration (dry mass basis)
Organic content	52
Inorganic content	48
Total carbohydrate	22
Crude protein	25
Elemental analysis C	18,7
H	4,7
N	3,2
Mg	9,8
Ca	9,5
K	6,4
P	14,5
Oxygen and the rest	33,2 (by difference)
Yield of polymer (dry mass g ℓ^{-1})	0,7 – 3,2
Molecular mass of polymer	2 x 10^6

- The suspended solids constitute some 18 per cent of the total solids of the factory waste which has a milky appearance, similar to a colloidal dispersion. The presence of suspended and colloidal particles, especially powdered carbon, could be important in terms of microbial attachment. In comparison, pelletisaton of sludge has so far been found with wastewaters containing little or no solids (Pol *et al.*, 1981).

- Starch, glucose and their derivatives are extensively used in the food, biotechnical and chemical industries as bonding, adhesive and agglomerating agents and also for their gel formation, encapsulation and film forming properties (Muller, 1975). To what extent these properties are exhibited in the wastewater and interact with the biomass to form pellet sludges, needs further investigation.

Pellet sludge formation on the full-scale clarigester plant

Start-up of the plant was achieved by initially filling the clarigesters with settled sewage and then inoculating with primary sewage sludge on a daily basis, according to the schedule in Figure 2. The addition of primary sludge was terminated after some 8 months at which stage a suspended solids concentration of 30 and 8 g ℓ^{-1} had accumulated in the bottom and middle sections of the digestion compartments, respectively.

Acclimatisation to glucose/starch feed was commenced after the sixth month by adding the wastewater together with the raw sludge. The initial space load rate due to the glucose/starch feed alone was approximately 0,2 kg COD m^{-3} d^{-1}, based on the volume of the digester compartment. This was equivalent to some 10 per cent of the factory flow and was progressively increased to the full flow of 500 m^3 d^{-1} over a period of two months (Figure 2). The decision to increase the feed flow rate was based on the efficiency of the biomass to metabolise the volatile fatty acids. The digester acids never increased above a concentration of 400 g ℓ^{-1} during the acclimatisation period. Thereafter, the plant has been continuously operated on glucose/starch waste alone.

Due to production schedules, the volume and character of the waste can change substantially during the course of the day. For example, the flow rate and COD concentration can vary in the range 400 - 900 m^3 d^{-1} and 1 - 20 g ℓ^{-1}, respectively. The digester compartment space load rates are for this reason maintained at a low level (2 kg COD m^{-3} d^{-1}) in order to prevent the

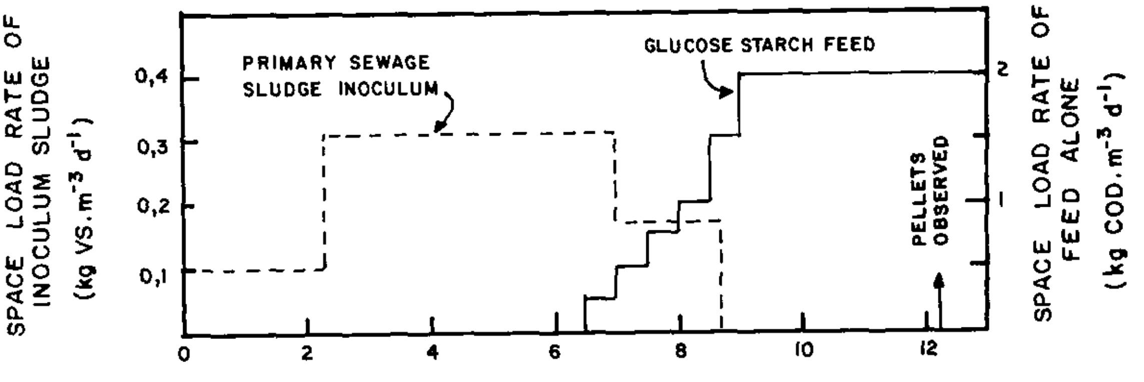


Figure 2
Schedule of start-up of clarigester plant

risk of overloading as a result of peak load values. It should be realised that in such a plug flow system the actual sludge bed load rate in proximity to the feed inlet is significantly higher than in other parts of the reactor.

The pelletisation of the sludge was first observed some 6 months after acclimatisation with the feed had commenced. At that stage many of the pellets were 3 – 4 mm in diameter and it is logical to conclude that initiation of the reaction had started much sooner. The operational conditions existing on the plant at that time have been summarized in Table 3.

Pellet sludge formation on the pilot-scale UASB plant

A pilot-scale UASB type reactor of 4,3 m^3 total capacity (Figure 3) was commissioned adjacent to the full-scale plant in order to study the sludge pelletisation process in more detail, and specifically at higher load rates. The sludge from the clarigesters was used as inoculum for this purpose. The major differences in the mode of operation of the two plants are that the feed to the pilot plant contains less suspended solids (due to balancing and settlement) and is furthermore preheated to 35 °C with steam.

TABLE 3
OPERATIONAL CONDITIONS OF GLUCOSE/STARCH DIGESTER COMPARTMENT UNDER WHICH PELLET SLUDGE IS MAINTAINED

Parameter	Full-scale clarigester plant	Pilot-scale UASB Plant
Space load rate (kg COD m^{-3} d^{-1})	2 – 3	10 – 20
Sludge load rate (kg COD kgVSS^{-1} d^{-1})	0,15	0,7
Hydraulic retention time (d)	3	0,4
Temperature (°C)	15 – 25*	35
Suspended solids in bed (g ℓ^{-1})	35	75
Volatile suspended solids (%)	90	90
Volume of digester compartment (m^3)	1 860	2,3
COD reduction (%)	93 – 95	93 – 95

*Ambient temperature.

Satisfactory treatment efficiency is obtained at digester space and hydraulic load rates of 10 – 20 kg COD m^{-3} d^{-1} and 0,4 days, respectively. This is significantly higher than that maintained on the full-scale plant. The improved performance of the UASB reactor resulted in the development of a pellet sludge with certain different features. These aspects are discussed in more detail below.

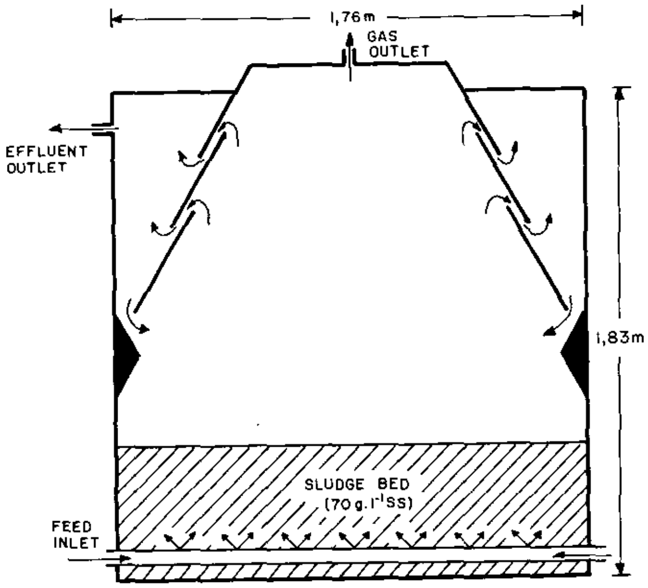


Figure 3
Pilot-scale UASB digester (total capacity of 4,3 m^3)

Pellet sludge characterisation

Size and shape of pellets

The pellets obtained from the full-scale clarigesters vary in size from large granules (7 mm) to very fine particles with different zone settling properties. These pellets have a spherical shape as illustrated in Figure 4. In comparison, the pellets obtained from the pilot UASB reactor are generally smaller but do not have such a wide range in size (0,5 – 2 mm). Furthermore, these pellets are more irregularly shaped, as is apparent in Figure 5.

Figure 4
Sludge pellets from the full-scale clarigesters (Grid size 1 mm²)

The periphery of the pellet surface in both instances is well defined and no filaments extend beyond the surface, as is the case with most sludge flocs. The agglutination reaction does not seem reversible as the pellets retain their shape and settling properties even after one year of storage without substrate.

Zone settling and thickening properties

The solids flux method was used to determine the zone settling transmissible capacity and thickening characteristics of the glucose/starch pellet sludge (Ross and Smollen, 1981). For this purpose, a series of settlement-time plots were made at different sludge concentrations using a 50 cm high stirred batch settling apparatus. A typical solids flux curve (Figure 6) for sludge sampled from the bed of the pilot plant reactor recorded a maximum solids flux value of 4500 kg.m^{-2} d^{-1} suspended solids. The flux curve predicted a potential sludge thickening to a concentration of some 90 g ℓ^{-1} suspended solids which was in agreement with the actual conditions existing on the pilot plant i.e. 75 g ℓ^{-1}. The stirred sludge volume index (SSV) on the same sludge recorded a value of 11 mℓ g^{-1} at 60 g ℓ^{-1} SS.

The good settling and thickening properties of pellet sludges

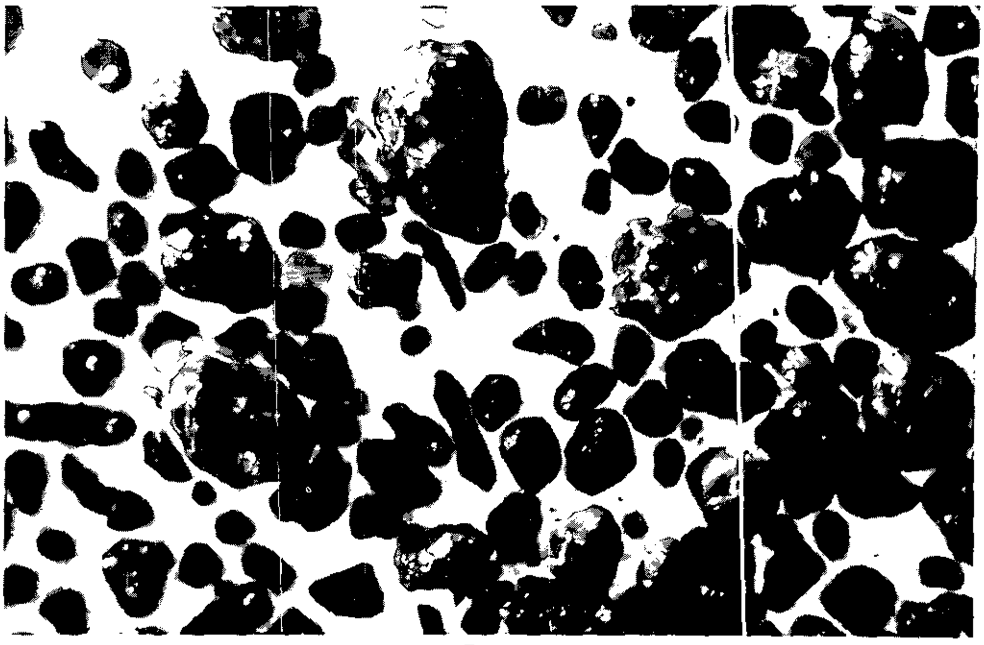


Figure 5
Sludge pellets from the pilot UASB reactor (Grid size 1 mm²)

have also been confirmed by Lettinga *et al.* (1979) and Pette *et al.* (1980) who reported the presence of a granular sludge bed with a total solids content of 100 – 150 g ℓ^{-1} at the bottom of the reactor.

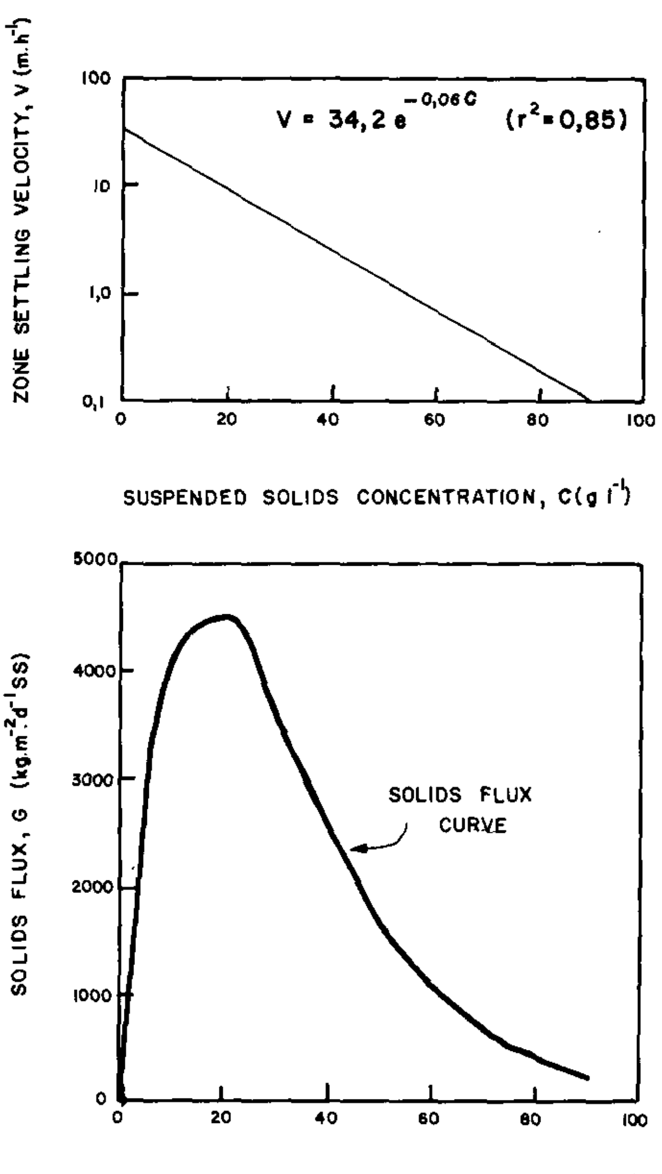


Figure 6
Solids flux curve of pellet sludge

Particle settling velocities

The rate of settling of the individual pellets was determined in a 2 m long by 0,18 m diameter computer-linked settling tube, according to the method used by Brink and Rogers (1979). After settling a distance of 2 m in water, the sludge pellets are collected in a flat pan which is attached to an electronic balance. During experimentation, cumulative mass readings are processed every 1,5 s by a desk-top calculator and plotter. The results of these settling tests on pilot plant pellets (size 1 – 2 mm) established that the rate of settling varied in the range 60 m h^{-1} for the fastest pellets to 6 m h^{-1} for the bulk of the slowest particles.

According to the determined settling curve, 50 per cent of the pellets settled faster than 27 m h^{-1}.

Chemical characteristics of sludge pellets

Various chemical analyses of the pellets are presented in Table 4. It was established that the percentage volatile suspended solids content of the pellets increased from an initial value of some 75 per cent to a value of 90 per cent after one year of digester operation. This is significantly higher than the 70 per cent generally reported for biological sludges. The low inorganic fraction of 10 per cent is still sufficient to give the pellet a high density so that it will settle quickly. Crude protein was found to constitute the major fraction of the sludge on a dry mass basis. This concentration agrees with the nitrogen value of 11 – 12,5 per cent, as reported by Lettinga *et al.* (1980). Total carbohydrates amounted to a further 10 – 12 per cent.

TABEL 4
CHEMICAL CHARACTERISTICS OF SLUDGE PELLETS

Parameters	Percentage Concentration (dry mass basis)
Organic content	90
Inorganic content	10
Nitrogen content	11
Crude protein	66
Total carbohydrate	11
Yield of ECP material	4
Molecular mass of polymer	2×10^6

Sludge extracellular polymers (ECP) were extracted from the washed pellets by boiling at a neutral pH for 1 hour followed by centrifugation and precipitation with 2 volumes ethanol per 1 volume centrate, according to the method of Forster (1971) as modified by Beccari *et al.* (1980). The yield of ECP by this method amounted to some 4 per cent of the suspended solids on a dry mass basis.

Of particular interest is the fact that a fraction of both the soluble polymer in the glucose/starch feed and the ECP which was bound to the pellet sludge surface have the same molecular mass, namely 2 million.

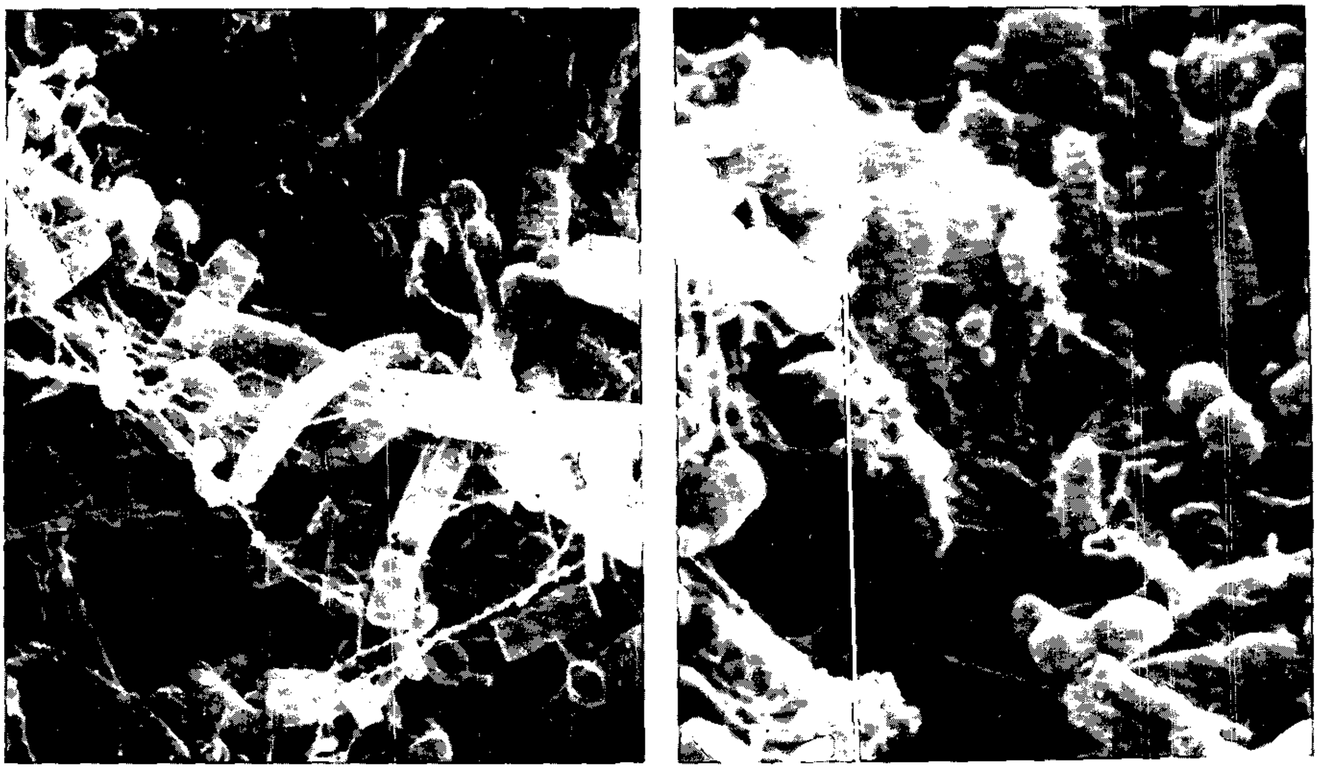
Microbiological characteristics of sludge pellets

Microscopic examination of the crushed pellet by wet and stained preparations reveals the presence of predominantly three morphological types of bacteria:

- Long rods (5-15 μm) with flat ends; non-motile; gram-positive.
- Cocci (2,5 μm diameter) often in small bunches of two to three bacteria; non-motile; gram-positive.
- Short rods (2,5 μm long); very motile; gram-negative.

The pellets probably consist of a consortium of anaerobic and facultative acidogenic, acetogenic and methanogenic bacterial populations. More basic studies on the microbiology of the pellet sludges are being undertaken by Steyn and Howgrave-Graham (1983).

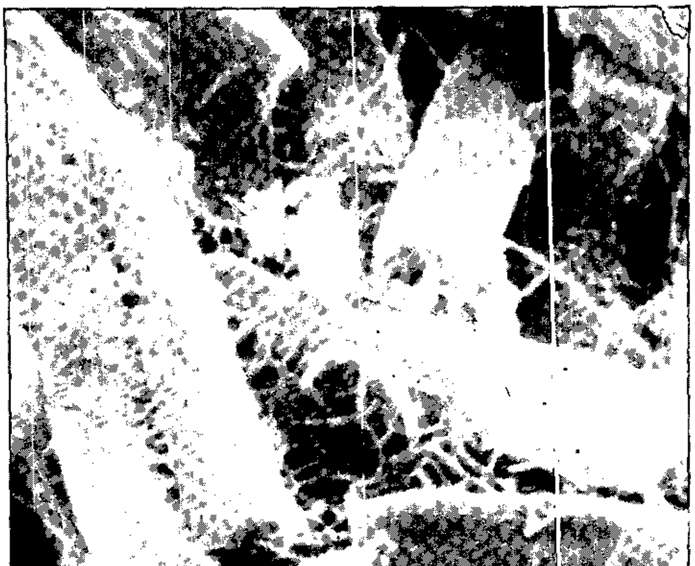
The bacterial morphology of the pellets was examined in greater detail by means of scanning electron microscopy. In order to preserve the morphology as representatively as possible, the pellets were fixed in gluteraldehyde and osmium, washed, dehydrated via a graded series of alcohols, followed by critical point



(a)

(b)

Figure 7
Scanning electron micrographs indicating the presence of extracellular polymer. The fribrous strands around the bacteria appear to hold the sludge pellet intact. (a) Magnification 8 000 x (b) Magnification 10 000 X (c) Magnification 20 000 X



(c)

drying. Magnification of 8000 times and higher revealed a variety of rod type and long filamentous bacteria and also the presence of ECP. It is evident from Figures 7 a, b and c that the bacterial cells are interwoven by a network of fibrous strands or appendages analogous to a cobweb, which appears to hold the pellet intact. Such fibrous strands are generally comprised of polysaccharides and are similar to those reported by Wheatley (1981), Paerl (1975) and Dugan and Pickrum (1972). The key feature in bioflocculation appears to be the synthesis of relatively insoluble ECP fibrous strands so that they remain in the vicinity of the cells and do not dissolve away.

The presence of insoluble polysaccharide strands around the bacteria was established by staining the crushed pellet with Alcian blue and counterstaining with Carbol Fuchsin according to the method of McKinney (1953). Using this specific stain, the insoluble polysaccharide strands and capsules appear as blue and the bacterial cells as red. Dugan and Pickrum (1972) were similarly successful using the fluorescent dye paper white under ultraviolet illumination. They reported that most of the cells could not be focussed because of the optical interference of the invisible polymer.

Possible mechanisms of pellet sludge formation

A perusal of the literature reveals that various researchers have been successful in cultivating pellet sludges (Lettinga *et al.*, 1979; Pipyn and Verstraete, 1979; Cohen *et al.*, 1979; Pette *et al.*, 1980; de Zeeuw and Lettinga, 1980; Zoetemeyer *et al.*, 1981). The phenomenon is however not widespread in wastewater treatment and an attempt was made to find some common denominator which could be responsible for the formation of the pellets. It would appear that well distinguishable anaerobic sludge pellets with good settling properties have mainly been formed in upflow sludge bed reactors and generally when treating a carbohydrate or volatile fatty acid type of waste. Notwithstanding the various studies that have been carried out, the actual mechanisms of anaerobic pelletisation are not very well understood.

In comparison, a considerable amount of detailed research has been undertaken on the mechanisms of biological flocculation in aerobic treatment systems (McKinney, 1952; Tenney and Stumm, 1965; Pavoni *et al.*, 1972; Forster, 1976, Brown and Lester, 1979). Most of the mechanisms for bioflocculation or agglutination are based on the complex interactions of high molecular mass extracellular polymers which are excreted or exposed under certain physiological conditions at the microbial surface. These naturally produced polymers bond electrostatically or physically and subsequently bridge the bacterial cells and other particulate materials into a multi-component matrix of sufficient magnitude to settle as floc aggregates. The slime and capsular material constituting ECP has been loosely described as mucilage (Takiguchi, 1968) and is also consistent with Costerton *et al.* (1979), describing a glycocalyx. It is however, uncertain at this stage to what extent these concepts are also relevant to anaerobic systems.

Sufficient evidence exists of the interrelationship between ECP accumulation and microbial agglutination (Brown and Lester, 1979). Furthermore, agglutination of bacteria is generally due to the interaction between a polysaccharide and a protein. In this context, it is considered significant that not only is ECP also largely composed of polysaccharides and proteins but that these organics constitute the major fraction of the glucose/starch substrate under study.

From the aforegoing discussion it appears feasible that the clumping of the bacteria in anaerobic pellet sludges is similar to an agglutination reaction as induced by polymers. These polymers can be present in the feed substrate as such (feed polymers) or are synthesised by the polymerisation of simpler precursor molecules as a result of the metabolism of the feed substrate (biopolymers).

Due to the excellent settling properties and size of the anaerobic sludge pellets, it would appear that the agglutination reaction is in this instance much more pronounced than is generally the case in conventional aerobic and anaerobic treatment systems. Furthermore, the design and operation of the upflow sludge bed reactors, with concomitant low shear mixing, play an important role in maintaining the pellet structure.

Conclusions

The successful cultivation of pellet sludges has important economic implications for the optimisation of biological treatment processes in terms of improved sludge settleability, biomass retention and high loading rates. Most of the studies reported in this paper have been very much of a preliminary nature, but have nevertheless provided experimental evidence of the role that feed polymers or extracellular polymers play in the bioflocculation of bacteria to form pellet sludges.

It is realised that the concept of sludge pelletisation would have limited application if the process was found to be dependent on the type of waste to be treated. For this reason, attempts will be made to induce the reaction into other waste treatment systems, once the mechanisms underlying the process have been established.

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