Bacterial identification of granular sludge from domestic sewage UASB-reactor

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SUMMARY

Species of non-methanogenic bacteria were isolated and identified in granular sludge from UASB reactors, treating domestic sewage: Butyrivibrio fibrisolvens, Clostridium sp, Desulfovibrio desulfuricans. These species are related to the hydrolitic and acetogenic phases and with the sulphate ion reduction in anaerobic digestion. It was also noticed bacteria similar to Desulfotomaculum. Two genus of methanogenic bacteria were isolated: Methanobacterium sp and Methanotrix sp. The granular sludge presented specific activity values about 0.15gCOD-CH_4/gVSS.d^{-1}, with spherical - (diameter about 4mm), resistant and dark granules.

INTRODUCTION

When the advanced anaerobic reactors were developed, the phenomena of cells agregation and biofilms forming over inert surfaces, became very important because they are fundamental characteristics of celular - retention inside the reactor. The best known examples are: the anaerobic filters, which packing material is used as a support for the bacterial biofilm; fluidized bed reactors, where sand acts as a support; UASB reactors where the conditions of the system provides

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cells granulation. The knowledge of factors that conduce biofilm and granules formation, has great interest in the reactor's control and operation. Particulary, granulation in UASB reactors has revealed the importance of biological, chemical and physical conditions to granules development. Regarding to the biological conditions, efforts were made to evidence the biological agents, which are responsible to the phenomena. Hulshoff Pol et al (1) reported the presence of Methanotrix and Methanosarcina in studies about factors affecting start-up and granulation in UASB reactors. Brummeler et al (2) and Dolfing et al (3) confirmed the dominant presence of a acetothrophic methanogenic of Methanotrix genus in digestors operating with and acetate-propionate mixture and with a sugar factory wastewater, respectively. Novaes et al (4) noticed the presence of a rod-shape - Methanotrix like in UASB reactors treating brewery - wastewaters. Beside the methanogenic species, the granules have also non-methanogenic bacteria and this study present the results of isolation and identification of these bacteria.

MATERIALS AND METHODS

1. Enrichment of the reactor:
   An 1L reactor was inoculated with spheric (diameter about 4mm), resistant and dark granules, originated from a UASB reactor treating domestic sewage (5). The culture medium was described by Karube et al (6) and temperature was controled in 30°C.
2. Composition of culture media to isolation procedures: by Salinitro et al (7)[1], for non-methanogenic' bacteria; by Postgate (8)-E, for reducing-sulphate - bacteria; by Bryant, for acetotrophic and hydrogenotrophic methanogenic bacteria; by Huser et al (9), - for acetotrophic methanogenic bacteria.
1. NOTE Media modified by CETESB'S laboratories (10).
3. Procedures for anaerobic manipulation: Anaerobic - technique described by Hungate (11) and Bryant (12), with modifications described in CETESB anaerobic technical manual (10).
4. Bacterial identification tests: morfological characteristics of colonies and bacterial cell; physiological characteristics - Gram stains of isolates, strict anaerobic conditions, gelatin liquefaction, casein hydrolysis, catalase and oxidase production, motility, growth in Blood Agar medium and utilised substrates. Separation of volatile fatty acids (acetic, propionic, isobutyric, butyric, isovaleric, valeric) and gases composition (H_2S, CH_4, CO_2) were achieved by cromatographic analyses.
5. Activity methanogenic test described by de Zeeuw (13).

RESULTS AND CONCLUSIONS

The presence of non-methanogenic and methanogenic bacteria, was verified in the isolation and identification of bacteria present in granules of sewage UASB-reactor. This suggest the formation of a microenviro-

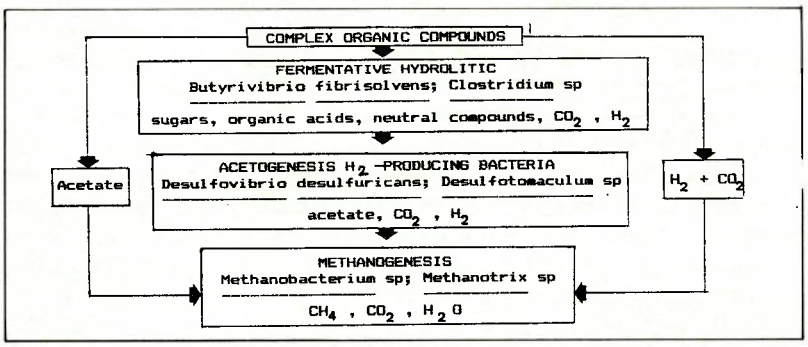

Fig. 1. Isolated Bacteria Groups and 3 steps of AD

TABLE 1. Routes of Compounds and Products Formation

1. Hydrolitic fermentative bacteria - cellulolytic:
   Cellulose → cellobiose + glucose → lactate + ethanol + acetate + CO_2 + H_2

   Butyrivibrio fibrisolvens
   Cellulose → cellobiose → butyrate + lactate + H_2 + CO_2
   glucose → butyrate + lactate + H_2 + CO_2

   Butyrivibrio fibrisolvens[1]
   Hexoses
   (cellobiose, glucose) → butyrate + ethanol + CO_2 + H_2O +2

   Clostridia
   Cellobiose → glucose I.P → ethanol + acetate + lactate + butyrate + formate + succinate + CO_2 + H_2
   glucose → ethanol + acetate + lactate + butyrate + formate + succinate + CO_2 + H_2

   Clostridia[4]
   Hexoses
   (cellobiose, glucose) → acetate + propionate + isobutyrate + isovaleric + ethanol + CO_2 + H_2O +2

2. Acetogenic H_2 - producing bacteria
   Sulphate - reducing bacteria (low $SO_4^=$):

   Desulfovibrio desulfuricans
   Lactate → acetate + CO_2 + H_2
   Organic acids → acetate + CO_2 + H_2

   Desulfotomaculum sp
   Lactate → acetate + CO_2 + H_2
   ethanol → acetate + CO_2 + H_2

   D. desulfuricans[1]
   Lactate + SO_4 → acetate + H_2S + (CO_2 + H_2O)[2]

3. Methanogenic bacteria
   Hydrogenotrophic - Methanobacterium sp[1]
   H_2 + CO_2 → CH_4 + H_2O
   Acetotrophic - Methanotrix sp[1]
   acetate → CH_4 + CO_2

Notes: 1: Studied bacteria.
2: Non identified products.

TABLE 2. Products Formation by the isolates bacteria

BACTERIA		PRODUCTS
Butyrivibrio fibrisolvens		butyric[2]
Clostridium sp[1]	cult 1	acetic, propionic, isobutyric, isovaleric[2]
	cult 2	acetic, propionic, isobutyric, isovaleric[2]
	cult 3	acetic, propionic, isobutyric, isovaleric[2]
	cult 4	acetic, propionic[2]
	cult 5	acetic, propionic, isobutyric, isovaleric
Desulfovibrio desulfuricans		acetic[2]

Notes: 1: differences between cultures are in the position of the spore and cell's size.
2: ethanol detection during acids analyses.

nment in the granule, where changes between many kinds of bacteria takes place, as it has been evidenced in the scheme of the biochemical steps of anaerobic digestion (14). Figure 1 shows the isolated bacteria - groups wich characterize the 3 steps of anaerobic digestion, hydrolysis and fermentation, acetogenesis - with H_2 production and methanogenesis. The results of the kinds of bacteria founded made possible the description of utilization routes of compounds and products formation in the reactor, according to data described in the literature (8, 15, 16) which are represented in Table 1. The organic compounds produced by non-methanogenic cultures (see Table 2), agree - with some of the products formation ways presented - in Table 1. The methanogenic phase was represented - by isolated and identified hydrogenotrophic bacteria of Methanobacterium genus, and by gram-negative, acetotrophic and filament forming bacteria, wich suggest the presence of Methanotrix genus. The specific methanogenic activity of the studied sludge, was about 0.15 $gCH_4COD/gVSS \cdot d^{-1}$, which was considered' a low value according to that founded for granulated sludge by de Zeew (13). So, we are now studying some modifications in the test to adapt it to sludges originated from sewage treating reactors, mainly in respect to initial acid concentration of medium culture.

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Section 1

ANAEROBIC DIGESTION FUNDAMENTALS

1.1. Microbiology and Biochemistry

1.2. Environmental Factors