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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Industrial Phytopesticide Wastewater Treatment using Methanogenic Consortium

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Abstract : Treatability of industrial phytopesticide wastewater collected from azadirachtin manufacturing unit in Tamil Nadu, India was investigated using a continuous anaerobic sludge bed reactor (ASBR). The reactor is a conglomeration of the positive features of an anaerobic filter (AF) and an upflow anaerobic sludge blanket (UASB) reactors. The inoculum was prepared using sewage sludge and cow dung. The reactor was operated at the ambient temperatures (30 – 35^{o}C) for 115 days. For the first 25 days, acclimatization was done at 24 h hydraulic retention time (HRT). From days 26 to 89, the reactor was operated at the organic loadings (OLs) of around 4000, 5000, 6500 and 7600 mg COD/L at 24 h HRT and the corresponding COD removal efficiencies were 82.65, 93.51, 96.0 and 57.77 %. It is, from the recorded values, inferred that 6400 mg COD/L was found to be the best suited initial substrate concentration for achieving greater reactor performance. In this period, the production of biogas ranged from 2800 mL/d to 7000 mL/d. With the increase in organic loading rate the biogas production rate was also increased. During 90 – 115 days, the OLR was increased from 12.8 to 25.6 kg COD/m^{3}d by decreasing the HRT from 12 h to 6 h and keeping the substrate concentration constant closely around 6400 mg COD/L. Scanning electron microscopic (SEM) observation of the granules showed the presence of *Methanosarcina* sp. and *Methanosaeta* sp. as the dominant species.
Keywords: Phytopesticide wastewater, Methanogenic consortium, Continuous anaerobic sludge bed reactor.

Introduction

Over the years the demand for energy has grown rapidly, due to increasing population, industrialization, urbanization, transportation and agriculture[1]. There is a continuous search for alternate energy sources to reduce the dependence on fossil fuels[2, 3]. In recent years, the biological degradation of organic wastes by anaerobic digestion has emerged as one of the major processes for the production of biogas[4]. The present work aims at developing a suitable technology to utilize wastewater from biopesticide industry for energy production.

Phytopesticide, azadirachtin has been identified as neem seed's chief ingredient. Its empirical formula is X_{35} H_{44} O_{16} and molecular weight is 720. It belongs to the chemical family of tetranortriterpenoids. During the biopesticide (azadirachtin) production, industrial biopesticide wastewater is mainly generated from extraction and solvent recovery units. Solvents such as n – hexane and ethyl acetate are used in the manufacture of biopesticide. It is, from the scanning of all the available literature, known that hitherto scant attention has been given to treat industrial biopesticide wastewater and no attempt has been made in using the ASBR. ASBR proposed over recent years has elicited considerable interest because of its greater removal efficiencies of organic substrates, its relationally simple layout and the low capital and operating costs[2].

Materials and Methods

Collection of sample

The wastewater was collected from phytopesticide manufacturing unit, Cuddalore, Tamil Nadu. The raw effluent collected was thoroughly mixed and the integrated sample was used for the study. All chemicals used were laboratory–grade reagents.

Anaerobic sludge bed reactor (ASBR)

The laboratory scale anaerobic sludge bed reactor (ASBR) used in this study was made from perpex tube with an internal diameter of 10.4 cm and an overall height of 60 cm. The top third of the reactor (10 cm) was filled with polypropylene spherical beads. This packed section performs dual functions of retaining the suspended sludge within the reactor and exerting a polishing effect on the wastewater through the activity of the bio-film developed on the packing material. At the lower part of the reactor an inlet was fixed. At the upper part of the reactor, above the packing column, outlet of the effluent was made. The outlet fixed on the topmost part of the reactor was meant for the flow of gas and gas flow meter was connected to it. The volume of the biogas collected was found out using the gas flow meter.

Start – up process

To begin with, the reactor was fed with the inoculum – a mixture of sieved sewage and cow-dung slurry (700 mg/L of suspended solids) at 24 h hydraulic retention time (HRT). This HRT facilitated the buildup of microorganisms on the packing medium and at the bottom. Following the recommendations of Mullai[5], acclimatisation media with the constituents reported in Table 1 was prepared. Then for 10 days the prepared acclimatisation media was passed into the reactor. The gas production was noticed at the fag end of this period. Thereafter, the feeding of basic feed was commenced.

Table 1 Acclimatisation media

S. No.	Parameters	Concentration (mg/L)
1	Glucose	1000
2	Urea	227
3	Magnesium sulphate	100
4	Ferric chloride-hexahydrate	0.5
5	Calcium chloride-dihydrate	0.7
6	Potassium-dihydrogen orthophosphate	527
7	Di-potassium hydrogen orthophosphate	1070

Table 2 Physicochemical characteristics of the phytopesticide wastewater

Parameters	Concentration – Average value, (mg/L)
pH	5.2 – 5.7
BOD	4000
COD	14,000
TDS	300
TSS	150
TS	2100

Experimental procedure

The characteristics of the phytopesticide wastewater used in this investigation are given in Table 2. The wastewater was diluted with tap water to a desired concentration. The pH was adjusted to 7.2 –7.4 by adding sodium bicarbonate before feeding. The reactor was operated continuously for 115 days in three phases.

Sampling and analysis

The sample was collected and analysed at once. The flow rate, pH, alkalinity, acidity, COD and biogas production were recorded daily and analysed by following the standard procedures given by APHA[6]. The microbial community present in the sludge granules that degrades the phytopesticides wastewater was found out using scanning electron microscope (SEM) (JEOL– JSM 5300, Japan).

Results and Discussion

Acclimatisation phase (Phase-I)

During this phase (0 – 25 days), acclimatisation was done by feeding the phytopesticide wastewater along with acclimatisation media. This process was carried out in order to acclimatise the biomass to phytopesticide wastewater. The glucose concentration of 1000 mg/L in the acclimatization media contributed to about 1067 mg COD/L[7]. During the course of operational period, the substrate concentration was gradually increased by subsequently decreasing the quantum of phytopesticide wastewater. As the phytopesticide wastewater used in this investigation contains solvents and other chemicals, it is likely that it may delay or prevent granular formation. Thus, to avoid such a situation glucose was added along with the substrate to facilitate faster microbial growth and in turn to speed up degradation process. Ghosh and Philip[8] in their work on atrazine degradation proved the role of external carbon source.

For the OLRs ranging from 1.3 to 2.8 kg COD/m^3d, the steady state value of COD removal efficiency was 86.43% recorded on 25th day of the experiment (Fig. 1). The highest COD removal efficiency of 86.43% achieved might be attributed to the longer residence time. The lowest COD removal efficiency of 28.51% achieved might be due to the active prevalence of acidogenesis at the start of the experiment and also due to limited time available for acclimation[2]. In acclimatisation phase, the values of effluent pH ranged between 7.8 and 8.2 (Fig. 2) indicated a healthy anaerobic environment[9].

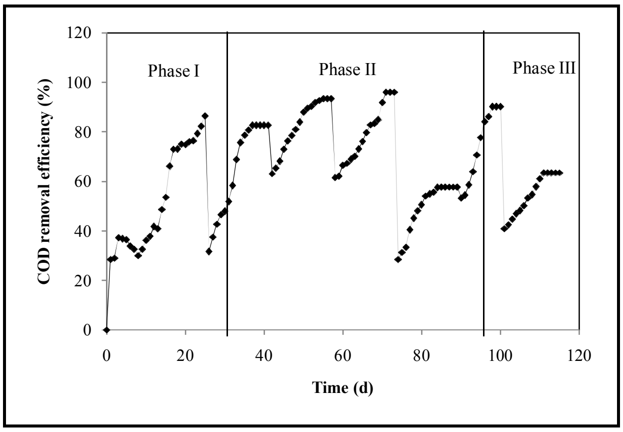

Fig. 1 Effect of time on COD removal efficiency in phases I, II and III

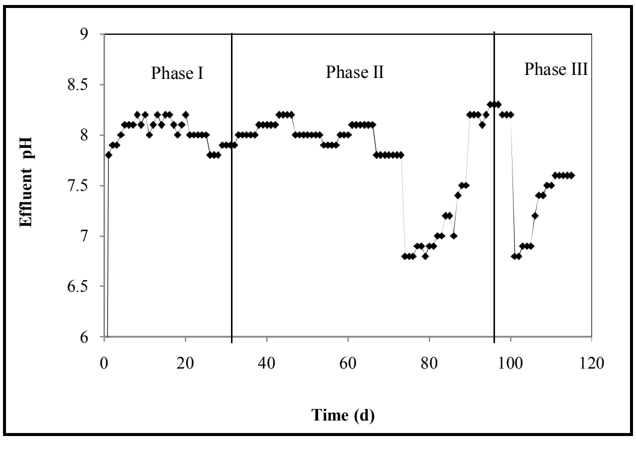

Fig. 2 Effect of time on effluent pH in phases I, II and III.

During this phase, the production of biogas ranged from 400 to 2600 mL/d, for the OLRs ranging 1.3 to 2.8 kg COD/m^{3}d, the steady state value of biogas production was 2600 mL/d (Fig. 3). The record of lowest amount of biogas during the first day of this phase could be due to poor growth of biomass[10]. Being the initial period, some time is naturally needed for the biomass, especially, for the methanogens to get acclimatised[11]. When the days started progressing, granulation getting developed and a gradual increase in biogas production was noticed[5]. Along with the biomass, glucose supplement was also one of the reasons for the increase in the biogas production in this phase[8].

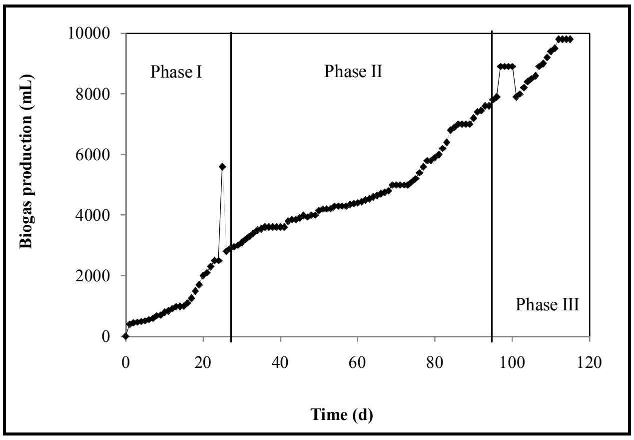

Fig. 3 Effect of time on biogas production in phases I, II and III.

Effect of initial substrate concentration (Phase-II)

In phase-II (26 – 89 days) the OLR was gradually increased from around 4.0–7.6 kg COD/m^{3}d, by increasing the initial substrate (influent) concentration from around 4000 to 7600 mg COD/L, respectively, and keeping the HRT constant 24 h. The COD removal efficiency which was higher (86.43%) on 25th day i.e., at the end of phase - I slashed to 31.73% on 26th day at the initial substrate concentration of 3999.8 mg COD/L and at the OLR of 3.998 kg COD/m^{3}d. Thereafter, the COD removal efficiency gradually increased towards the end of this OLR and reached a steady state value of 82.65% (Fig. 1). The fall in COD removal efficiency at the beginning of this phase may be attributed to the curtailment of glucose and temporary stress caused on the biomass due to sudden change in feed concentration[7]. When the OLRs were further increased to around 5.0 and 6.5 kg COD/m^{3}d by increasing the substrate concentration to 5000 and 6500 mg COD/L, respectively, at 24 h HRT on days 42 and 58, the COD removal efficiencies were found to decrease at the beginning of each OLR and gradually increased. This might be due to the increased initial substrate concentration needed sufficient acclimatisation period for the microbial consortium to acclimatise to the changed environmental conditions of the reactor[12]. The steady state values of COD removal efficiency for the above said OLRs were 93.51 and 96.09% respectively.

The increased COD removal efficiency might be due to formation of well acclimatised stable granular biomass owing to more nourishment and effective mixing due to upward escape of the produced biogas[13]. When the OLR was further increased to around 7.6 kg COD/m^{3}d by increasing the substrate concentration to 7600 mg COD/L, the reactor performance decreased and the steady state COD removal efficiency dropped to 57.77%. The decline in COD removal efficiency could be attributed to substrate inhibition as reported by Boopathy and Tilche[14] and Paula and Foresti[15]. From the results it is evident that the substrate concentration of around 6500 mg COD/L is the best suited initial substrate concentration for achieving a greater reactor performance.

During phase – II, the ranges in the values of acidity, pH and alkalinity were 196 –1087.8 mg acetic acid/L, 6.8 – 8.2 and 695 – 1397 mg $CaCO_3$/L, respectively. The values of acidity were higher at the beginning of each of the four OLRs, namely, 4.0, 5.0, 6.5 and 7.6 kg COD/m^{3}d, at 24 h HRT on 26, 42, 58 and 74th day and dropped to lower levels at the end of each OLR. A gradual fall in the acidity values at the end of each OLR may be to the existence of well acclimatised biomass which enabled greater COD removal efficiency, Augoustinos et al.[16] in their work on petrochemical wastewater made similar observations.

When the reactor was operated at around 7.6 kg COD/m^{3}d at 24 h HRT between 74 and 89 days, a higher acidity value of 1225 mg acetic acid/L was recorded at the beginning. The possible reason for such a higher acidity value may be attributed to greater feed concentration of around the 7600 mg COD/L. According to Campos and Anderson[17], increase in feed concentration leads to change in environmental condition within the reactor. As methanogens are highly sensitive, they are unable to cope–up with the abrupt changes in the reactor environment and become less active. But such a situation might have been taken advantage of by the acidogenic bacteria. Thus, as the concentration increased to 1225 mg acetic acid/L the pH and alkalinity values decreased to 6.8 and 695 mg $CaCO_3$/L, respectively, on 74th day. Consequent to this phenomenon, on 74th day the COD removal efficiency also decreased to 28.43%. In all the cases, the effluent alkalinity values were greater than the influent alkalinity during treatment. This increase was mainly caused by the mineralisation of protein into ammonia. The latter combine with the carbonic acid in solution to form ammonium bicarbonate buffer[18].

In phase – II, the production of biogas ranged from 2800 mL/d to 7000 mL/d (Fig. 3). As the organic loading rate increased, the biogas production also increased[8]. A marked increase in the production of biogas was also noticed along with increase in days as sketched in Fig. 3. Among all the OLRs, 7.6 kg COD/m^3d, the biogas production was so high and ranged from 5100 to 7000 mL/d between 74 and 89 days. The possible reason for such a relatively larger biogas production could be due to the enhanced feed concentration of 7600 mg COD/L. In their work on herbal pharmaceutical wastewater Nandy and Kaul[19] reported similar trends.

Effect of hydraulic retention time (Phase-III)

In this phase (90 – 115 days), the OLR was increased from 12.8 to 25.6 kg COD/m^3d by decreasing the HRT and keeping the substrate concentration constant closely around 6400 mg COD/L. The trend of percent COD removal efficiency as time progresses in phase – III is shown in Fig. 1. At 12 h HRT, the COD removal efficiency on the first day of this phase (90th day) was 53.25% as shown in Fig. 1 As day passed on, the COD removal efficiency increased gradually and reached a peak value of 90.20% on 100th day. When the HRT was brought down to 6 h with the corresponding OLR of 25.6 kg COD/m^3d on 101st day obviously a clear fall in the COD removal efficiency was noticed. As time progressed the COD removal efficiency gradually improved and attained the steady state value of 63.52%. The COD removal efficiencies decreased as HRT decreased. The lowering of HRT led to shorter residence time and resulted in limited degradation of organic matter. Similar trend of fall in the COD removal efficiency concomitant to decrease in hydraulic retention time was documented by Yu et al.[20]. At 6 h HRT with an OLR of 25.6 kg COD/m^3d, the lowest ever COD removal efficiency of 63.52% was achieved on 115th day. This could be because of disintegration and washing away of granular biomass along with the effluent due to high mixing intensities[13] with the highest flow rate. Augoustinos et al.[16], in their research on petroleum waste using the HUASB reactor, made a similar observation. This phase conclusively demonstrated that the ASBR used in this study could treat industrial biopesticide wastewater at higher organic loading rates of 12.8 kg COD/m^3d, with 90.20% of COD removal efficiency.

In this phase, the values of acidity, pH and alkalinity were in the ranges of 426 – 1000 mg acetic acid/L, 6.8–8.3 and 1225–2265 mg $CaCO_3$/L, respectively. In this phase, the quantum of biogas production fluctuated widely from 7200 mL/d to 9800 mL/d (Fig. 3). When the HRT was decreased from 12 h to 6 h corresponding to the OLR of 12.8 to 25.6 kg COD/m^3d, respectively, the biogas production rate decrease from 8900 to 7900 mL/d on 101st day, thereafter it started increasing. Saritpongteeraka and Chaiprapat[21] and Zhu et al.[22] reported the similar trend. The increase liquid velocity caused the bed expansion followed by the sludge washout which decreased the biogas production rate reached 9800 mL/d at the end of the 6 h HRT on 115th day. This finding corroborates well with the results obtained by Nandy and Kaul[19] in their work on herbal pharmaceutical wastewater.

Microstructure of granules

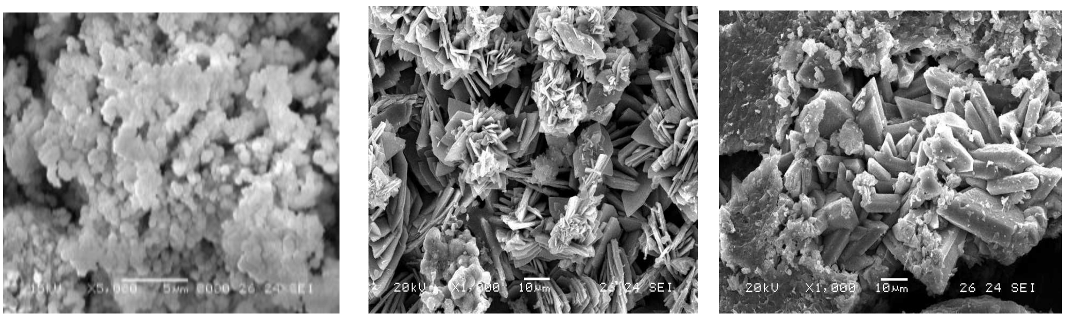

Fig. 4 SEM micrographs of *Methanosarcina* sp., *Methanosaeta* sp. and crystal like *Methanobacteria*

To elucidate, the morphology and microstructure, especially, to understand the pore structure, the granules colonization in the reactor was examined under a scanning electron microscope (SEM). The species were identified with the help of morphological features from the scanning electron micrograph and with that of the key characteristics from the literature[23]. Three species of methanogens sp. namely *Methanosarcina* sp., *Methanosaeta* sp. and *Methanobacterium* sp. were identified (Fig. 4). As reported by Sekiguchi et al.[24] under mesophlic conditions *Methanosaeta* sp. plays an important role in the formation of pores of the sludge granules which facilitate the passage of nutrients and for the escape of biogas. *Methanobacteriaceae* and *Methanosaeta* were found the main methanogens in a laboratory scale up-flow anaerobic digester treating olive mill wastewater[25].

Conclusions

The present investigation was carried out to assess the performance of the ASBR in treating phytopesticide wastewater. The quantum of biogas production increased with an increase in initial substrate concentration and with decrease in HRT. For the different initial subtrate concentrations, such as around 2500, 4000, 5000, 6500 and 7600 mg COD/l, it could be inferred that the initial substrate concentration of 6500 mg COD/l is the best suited to achieve greater reactor performance at 24h HRT. By varying the HRT, from 24h to 6h, it is concluded that the ASBR could treat phytopesticide wastewater effectively at the loading rate of 12.8 Kg COD/m^3d at 12 h HRT with the COD removal efficiency of 90.2%. Three species of anaerobic bacteria such as *Methanosarcina* sp., *Methanosaeta* sp. and crystal like *Methanobacterium* sp. were identified.

References

1. Lalov I, Bozadzhiev O. Biomethanation of wastewater from wood fiberboard industry in anaerobic multichamber reactor. J Chem Tech Metallurgy, 2014, 49: 133-138.
2. Mullai P, Arulselvi S, Ngo HH, Sabarathinam PL. Experiments and ANFIS modelling for the biodegradation of penicillin-G wastewater using anaerobic hybrid reactor. Bioresource Technol., 2011, 102: 5492-5497.
3. Mullai P, Yogeswari MK, Sridevi K. Optimisation and enhancement of biohydrogen production using nickel nanoparticles – A novel approach. Bioresource Technol., 2013, 141: 212-219.
4. Mullai P, Vivekanandan B, Karunanithi T, Sabarathinam PL. Modeling in a hybrid up flow anaerobic sludge blanket reactor. Poll Res., 2005, 24: 383-388.
5. Mullai P. Industrial wastewater treatment using a HUASB reactor, Ph. D thesis, Annamalai University, Annamalai Nagar, 2002.
6. APHA. Standard methods for the examination of waste and wastewater, American Public Health Associations, New York, 1995.
7. Tay JH, He YX, Yan YG. Improved anaerobic degradation of phenol with supplemental glucose. J Environ Eng., 2001, 127: 38-45.
8. Ghosh PK, Philip L. Atrazine degradation in anaerobic environment by a mixed microbial consortium. Wat Res., 2004, 38: 2277-2284.
9. Ligero P, Soto M. Sludge granulation during anaerobic treatment of pre-hydrolysed domestic wastewater. Water SA, 2002, 28: 307-311.
10. Mullai P, Yogeswari MK, Sridevi K, Ronald Ross P. Artificial neural network (ANN) modeling for hydrogen production in a continuous anaerobic sludge blanket filter (ASBF). Int J Applied Sci., 2013, 5: 1-7.
11. Tawfik A, Elsayed H, Dessouki, H. Hydrogen and methane production from starch wastewater in a mesophillic anaerobic baffled reactor. 17th International water technology conference, 2013.
12. Rao AG, Naidu GV, Prasad KK, Rao NC, Mohan SV, Jetty A, Sarma PN. Anaerobic treatment of wastewater with high suspended solids from a bulk drug industry using fixed film reactor (AFFR). Bioresource Technol., 2005, 96: 87-93.
13. Lettinga G, Van Velsen AFM, Hobma SW, de Zeeuw W, Klapwijk A. Use of upflow sludge blanket (USB) reactor concept for biological wastewater treatment especially for anaerobic treatment. Biotechnol Bioeng., 1980, 22: 699-734.
14. Boopathy R, Tilche A. Anaerobic digestion of high strength molasses wastewater using hybrid anaerobic baffled reactor. Water Res., 1991, 25: 785-790.
15. Paula DR, Foresti E. Kinetic studies on a UASB reactor subjected to increasing COD concentration. Wat Sci Technol., 1992, 25: 103-113.

16. Augoustinos MT, Britz TJ, Tracey RP. Anaerobic digestion of a petrochemical effluent using an anaerobic hybrid digestor. Biotechnol Lett., 1989, 11: 369-374.
17. Campos CMM, Anderson GK. The effect of the liquid upflow velocity and the substrate concentration on the start-up and steady-state periods of lab-scale UASB reactors. Wat Sci Technol., 1992, 24: 41-50.
18. Isik M, Sponza DT. Effects of alkalinity and co-substrate on the performance of an upflow anaerobic sludge blanket (UASB) reactor through decolorization of congo red azo dye. Bioresource Technol., 2005, 96: 633-643.
19. Nandy T, Kaul SN. Anaerobic pre-treatment of herbal based pharmaceutical wastewater. Water Res., 2001, 35: 351-362.
20. Yu J, Chen H, Ji M, Yue PL. Distribution and change of microbial activity in combined UASB and AFB reactors for wastewater treatment. Bioprocess Eng., 2000, 22: 315-322.
21. Saritpongteeraka K, Chaiprapat S. Effects of pH adjustment by parawood ash and effluent recycle ratio on the performance of anaerobic baffled reactors treating high sulfate wastewater. Bioresource Technol., 2008, 99: 8987-8994.
22. Zhu GF, Li JZ, Wu P, Jin HZ, Wang Z. The performance and phase separated characteristics of an anaerobic baffled reactor treating soybean protein processing wastewater. Bioresource Technol., 2008, 99: 8027-8033.
23. Salle AJ. Fundamental principles of bacteriology. Tata McGraw-Hill Publishing Company Ltd., New Delhi, 1997.
24. Sekiguchi Y, Takahashi H, Kamagata Y, Ohashi A, Harada H. In situ detection, isolation, and physiological properties of a thin filamentous microorganism abundant in methanogenic granular sludges: a novel isolate affiliated with a clone cluster, the green non-sulfur bacteria, subdivision I. Appl Environ Microbiol., 2001, 67: 5740-5749.
25. Rizzi A, Zucchi M, Borin S, Marzorati M, Sorlini C, Daffonchio D. Response of methanogen populations to organic load increase during anaerobic digestion of olive mill wastewater. J Chem Technol Biot., 2006, 81: 1556-1562.

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