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Environmental Protection Agency
An Ghníomhaireacht um Chaomhnú Comhshaoil

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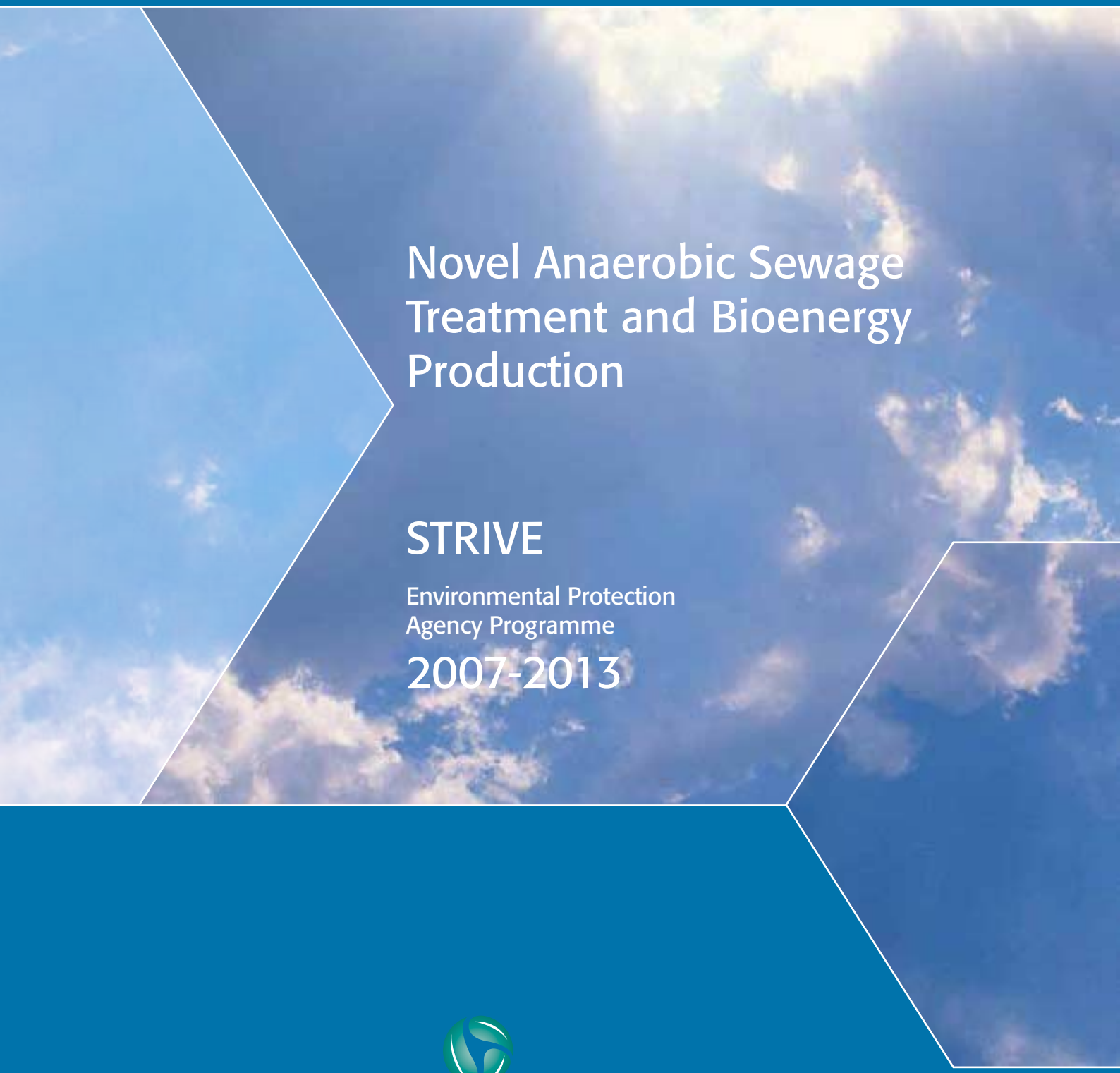

Novel Anaerobic Sewage Treatment and Bioenergy Production

STRIVE

Environmental Protection Agency Programme

2007-2013

SSTI

Comhshaol, Oidhreacht agus Rialtas Áitiúil
Environment, Heritage and Local Government

Environmental Protection Agency

The Environmental Protection Agency (EPA) is a statutory body responsible for protecting the environment in Ireland. We regulate and police activities that might otherwise cause pollution. We ensure there is solid information on environmental trends so that necessary actions are taken. Our priorities are protecting the Irish environment and ensuring that development is sustainable.

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EPA STRIVE Programme 2007–2013

Novel anaerobic sewage treatment and bioenergy production

High-rate Anaerobic Digestion as a Core Technology for Sustainable Treatment of Municipal and Low-strength Industrial Wastewaters

(2005-ET-MS-29-M3)

STRIVE Report

Prepared for the Environmental Protection Agency

by

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The project, entitled 'High-rate anaerobic digestion as a core technology for sustainable treatment of municipal and low-strength industrial wastewaters' was led by the National University of Ireland (NUI), Galway with collaborative support from international colleagues in the University of Michigan, USA (Prof. Lutgarde Raskin) and United Nations Educational, Scientific and Cultural Organization/ International Institute for Hydraulic and Environmental Engineering in Delft, The Netherlands (Prof. Piet Lens). The authors would like to thank Pat McCarthy at Carberry Milk Products (Ballineen, Co. Cork, Ireland) and the staff at the Mutton Island Sewage Wastewater Treatment Plant in Galway city for their full cooperation with the project and for the provision of samples. The support of Dr Michael Rodgers and his colleagues in the Discipline of Civil Engineering, NUI, Galway, with respect to phosphate analysis is also gratefully acknowledged. The Technology Transfer Office of NUI, Galway (Dr Neil Ferguson), Enterprise Ireland (Dr James Walsh) and Alan Phelan and the staff of Tethra-PM have been very supportive in the process of protecting the IP (No. 10193829.8 'An anaerobic reactor for psychrophilic and/or mesophilic wastewater treatment') generated during this project and in preparations for future commercial applications of low-temperature anaerobic digestion – the authors are very grateful for this support.

DISCLAIMER

The EPA STRIVE Programme addresses the need for research in Ireland to inform policy-makers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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Executive Summary

1.1 Background

The currently applied paradigm for municipal wastewater treatment in the European Union does not meet basic sustainability criteria. Indeed, it runs counter to the stated goals of recent European Council policies regarding sustainable development.[1] This disposal-based linear system uses aerobic microbiology as the core technology. This results – in the case of activated sludge plants, for example – in a requirement for large capital investment, heavy usage of fossil fuels, high-technology operational control and the generation of large quantities of sludge requiring treatment before safe reuse/recycle. Anaerobic digestion (AD) is a biological process of waste and wastewater treatment, which converts organic matter to biogas (a usable fuel) and a renewable energy source. The process occurs in bioreactors, where the microbes that carry out the process are retained as biofilms. Anaerobic digestion is an established, sustainable waste-treatment technology for residues from various sources, including industrial processes and agriculture. In addition to the generation of renewable energy, AD also has advantages over the conventional aerobic treatment approach, including lower capital and operating costs. To date, AD has not been applied for direct treatment of municipal wastewaters or domestic sewage in regions with a temperate climate, due mainly to concerns regarding the stability and efficiency of low-temperature AD. Recent advances in AD research and technology, however, have enabled high-rate, low-temperature anaerobic digestion (LTAD) as a feasible and potentially highly efficient approach. If proven feasible, an innovative municipal wastewater treatment approach, with AD as the core technology, could realise a major commercial and technological opportunity and facilitate future sustainable development in Ireland. This 36-month project evaluated, at laboratory scale and using state-of-the-art methodologies, the applicability and underlying microbiology of LTAD for municipal wastewater treatment under Irish conditions.

1.2 Research Methodology

This project was based on the design and operation of laboratory-scale anaerobic treatment systems. The bioreactors (3.5 l) were inoculated with anaerobic sludge granules obtained from a full-scale, internal circulation (IC) bioreactor at Carberry Milk Products (Ballineen, Co. Cork, Ireland). A hybrid bioreactor configuration with biomass immobilised both as granular biofilms and on media as fixed-films was chosen for the trials. For the first trials based on synthetic wastewaters, the fixed-film section of the bioreactors was packed using polyethylene rings (Ø, *c.* 25 mm), randomly packed in a cylindrical, wire-mesh cage. Subsequently, for trials on raw and settled sewage, a novel reactor configuration was used. In addition, an external membrane filtration unit was employed to polish the effluent from the bioreactors during some trials.

The bioreactors were employed to treat synthetic sewage and raw and primary settled sewage from the Mutton Island Wastewater Treatment Plant in Galway city in three long-term trials, ranging from 150–300 days in duration. During the trials, the operational conditions of the bioreactors were altered to establish the temperature and loading rate ranges applicable for successful wastewater treatment. In addition, biomass was sampled from the bioreactors and used to evaluate the development of active biomass under low-temperature conditions and to determine the physiological status of the microorganisms in response to changing operational conditions.

1.3 Research Findings

- A novel laboratory-scale bioreactor was designed and employed for LTAD of sewage. The process was applied successfully at organic loading rates (OLR) of up to 6 kg chemical oxygen demand (COD) $metre^{-3}$ day^{-1} and hydraulic retention times of 1.5 hours, using synthetic sewage, with average and stable total COD removal efficiencies of 70% under

1 http://ec.europa.eu/environment/eussd/

these conditions. A discharge standard, in terms of COD quality (125 mg l^{-1}) was routinely achieved using the systems for treating raw sewage from the Mutton Island plant.

- An active, low-temperature-adapted biomass was developed in the bioreactors, with a high level of microbial biodiversity, enabling a robust and stable process in response to changing environmental and operational conditions.
- Stable methanogenic populations were successfully maintained in the bioreactors, suggesting that the long-term operation of LTAD is completely feasible from a microbiological point of view.
- High levels of phosphate attenuation were achieved (up to 80% P removal) by the novel bioreactors, but the exact mechanism involved remains unclear.
- The potential biogas yield from LTAD of sewage, based on the results of this project, ranges between 0.1 and 0.26 m^3 per person per day, depending on dilution. The energy produced, therefore, from a 1000-person equivalent (p.e.) LTAD sewage treatment plant is the equivalent of 50–130 l of diesel per day (18–45,000 l/annum), which is available for use in the other wastewater treatment processes (drying, nutrient removal, disinfection, etc.); or for community use as vehicle fuel, household heating or power generation. In addition, and importantly, the displacement of heavy fossil fuel-utilising activated sludge plants with LTAD systems would give significant additional energy and greenhouse gas benefits (the water industry is the fourth most energy intensive sector in the UK; www.statistics.gov.uk*)*.

1.4 Commercialisation

In order to progress towards implementation of LTAD, the process must be demonstrated at a scale convincing to the water industry and policy-makers. An Invention Disclosure Form was lodged with the National University of Ireland (NUI), Galway, Technology Transfer Office in September 2009 on the novel low-temperature bioreactor process designed at NUI, Galway, and employed during this project. An application for European patent protection for the system was filed by NUI, Galway in November 2010. Funding has also been sought to construct a 150–200 p.e. mobile demonstrator-scale AD plant, and the research team is hopeful of a successful outcome. The new demonstrator plant should be installed at the Environmental Protection Agency Tuam Water Research Facility in Co. Galway and at a number of industrial sites in early 2011.

1 Introduction

1.1 Anaerobic Digestion

Anaerobic digestion (AD) is the biological mineralisation of organic matter to biogas, in the absence of oxygen, by the sequential activity of a number of microbial groups (Fig. 1.1a). Anaerobic digestion is an established waste-treatment technology for residues from various sources, including industrial processes and agriculture (Lettinga et al., 2001; McHugh et al., 2003a). It has advantages over aerobic treatment, including lower operating costs and, primarily, biogas production. Biogas is similar to natural gas and consists of a mixture of 50–85% methane and 15–50% carbon dioxide with trace amounts of other gases. It is a renewable energy source and is used to generate heat and electricity, process steam or as a vehicle fuel. The anaerobic reactors used for full-scale wastewater treatment and biogas production are high-rate systems based on immobilised or granular biofilms (anaerobic sludge; O'Flaherty and Lens, 2003; Fig. 1.1b), for example, the upflow anaerobic sludge blanket (UASB), or internal circulation (IC) reactors. The increased application of AD could provide benefits, both economically, by reducing energy and operational costs, and environmentally, by preserving fossil fuels and reducing polluting emissions. When compared to aerobic technologies, the anaerobic approach is advantageous with respect to its environmental and economic impact – low operating costs, compact construction, low surplus sludge production and, primarily, the *net production of energy* as biogas (Lettinga, 1995; McHugh et al., 2003a).

1.2 The Microbiology of Anaerobic Digestion

Despite the widespread harnessing of AD in engineered systems, knowledge gaps remain regarding the nature and function of the microbial populations involved in the process, including the mesophilic, sub-mesophilic or psychrophilic anaerobic digesters used for the treatment of domestic wastewaters (Lettinga et al., 2001; O'Flaherty and Lens, 2003). Historically, knowledge gaps relating to the microbiology of AD have resulted in a lack of integration of the fundamental processes occurring at microorganism level (scale *c.* 1 μm–1 mm) into the processes occurring within bioreactors (scale >1 m). Anaerobic digesters are mostly operated as 'black boxes', taking the effluent concentration as an output value that cannot be improved; and the process control strategy, if applied at all, does not generally take into account processes occurring at microorganism level. The result is that reactors are mainly designed using empirical design criteria, which can lead to the over-dimensioning of reactor volumes and sub-optimal or unstable treatment, an important feature with respect to anaerobic treatment of domestic wastewater. In some cases, the principles of design and operation of reactors can be derived from, for example, chemical engineering. However, there is a lack of systematic information about the nature and role of anaerobic consortia and the formation and growth of methanogenic biofilms and bioaggregates, which affects the design and operational stability of the systems (Wilderer et al., 2002). This is partly caused by the complex relationship between wastewater characteristics, process conditions and microbiology.

The lack of reliable and quantitative information on the microbiology of AD can be ascribed, to some extent, to technical limitations. Owing to the wide variety of starting products, a complex array of microbial species are involved in the AD process (Ferry, 1999), including some obligately syntrophic organisms (Schink, 2002). This has limited the value of traditional microbiological methods greatly. Additional information has recently been obtained, however, using molecular and microanalytical approaches, on the nature of anaerobic consortia and on the limitations and potential of the microbes involved (Schink, 2002; Collins et al., 2003, 2004a, b; McHugh et al., 2003a, b; 2004, 2005; Pender et al., 2004, Collins et al., 2006, McKeown et al., 2009). These new approaches have generated opportunities to improve the current state-of-the-art of anaerobic treatment, and increase possibilities to further broaden the application base of the technology.

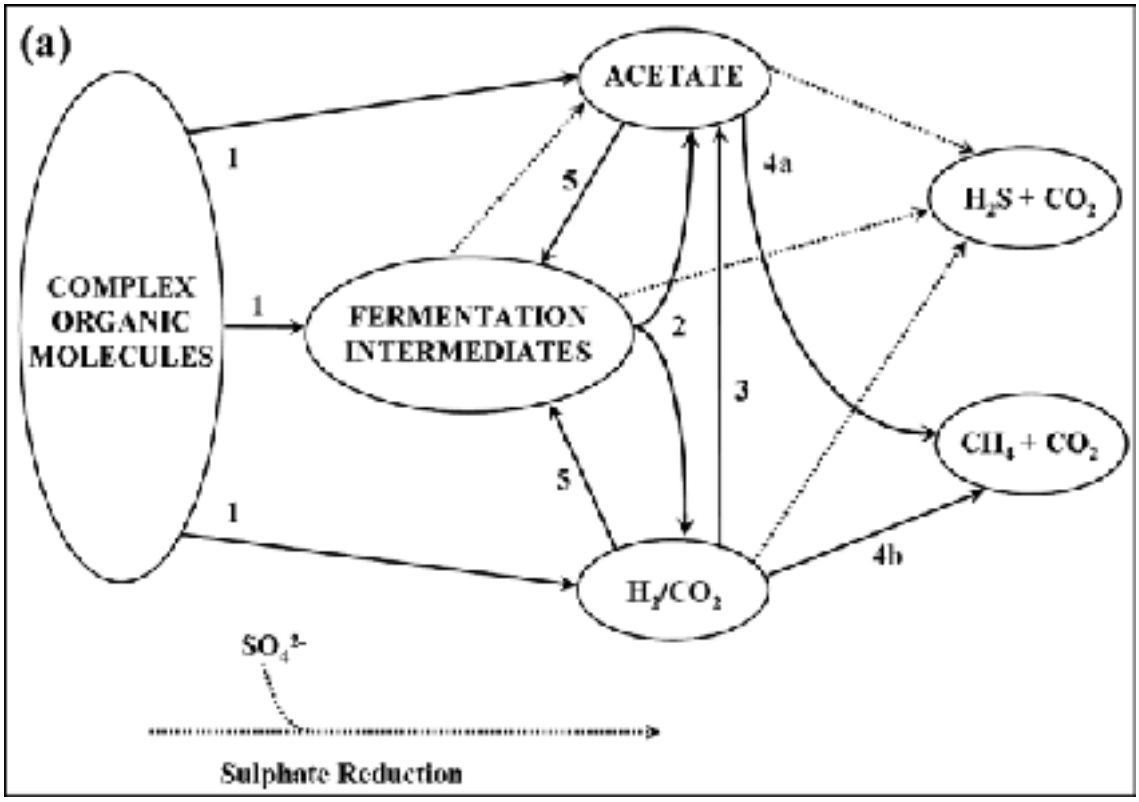


Figure 1.1. (a) Carbon flow in anaerobic reactors: 1 = hydrolytic/fermentative bacteria; 2 = obligate hydrogen-producing bacteria (syntrophs); 3 = homoacetogenic bacteria; 4a = acetoclastic methanogens (*Archaea*); 4b = hydrogenophilic methanogens (*Archaea*); 5 = fatty acid synthesising bacteria. Also shown (in broken lines): sulphate-reducing bacteria compete for electrons with methane-producing bacteria in the presence of sulphate. (b) Granular anaerobic sludge, scale bar 5 mm.

1.2.1 Methanogenesis

In the absence of electron acceptors, such as sulphate or nitrate, AD of organic matter occurs by the sequential cooperative action of a number of different bacterial trophic groups (Fig. 1.1a; Zeikus, 1982; Ferry, 1999; Lokshina and Vavilin, 1999). These microorganisms cooperate sequentially in order to achieve the degradation of a variety of polymeric and monomeric substrates. Digestion is initiated by the action of facultative and obligate fermentative bacteria, whose enzymes facilitate the hydrolysis of the initial proteins and polysaccharides, including the suspended organics present in domestic sewage, to monomeric sugars, amino acids, long-chain fatty acids and alcohols (Zeikus, 1979; Britz et al., 1994; Fig. 1.1). Further fermentation of the monomeric products by these, and other non-hydrolytic fermentative bacteria, results in the generation of a wide variety of fermentation end-products, including acetate, formate, methanol, H_2 and CO_2 (Fang et al., 2002). This initial fermentative activity is known as 'acidogenesis'.

The products of acidogenesis are further oxidised to acetate, hydrogen and carbon dioxide, in a process referred to as 'acetogenesis' (Schink, 1992; Stams, 1994), and mediated by the obligate hydrogen producing acetogens (OHPA). Under standard conditions, the oxidation of substrates such as butyrate, propionate or ethanol, to acetate, hydrogen and/or formate, or acetate, hydrogen/formate and carbon dioxide, is an endergonic reaction (e.g. $\Delta G^{\circ\prime}$ for propionate oxidation is +76.1 kJ/reaction; Table 1.1). It is only when the hydrogen partial pressure is lowered, for example, by the presence of hydrogen or formate-utilising methanogens, that the reaction becomes exergonic (e.g. the $\Delta G^{\circ\prime}$ for propionate oxidation is -25.6 kJ/reaction in the presence of a hydrogen-utilising methanogen). Therefore, OHPA bacteria must always grow in syntrophy with hydrogen-utilising methanogens, sulphate-reducing bacteria (SRB), or homoacetogens, in order to facilitate interspecies hydrogen transfer and gain energy from growth on the products of the acidogenesis phase (Schink, 1992; Li et al., 1994). Many syntrophic associations have been described in the literature (Stams, 1994; Schink, 2000, 2002), with optimum temperatures and pH levels for growth of between 25°C and 45°C and 6.3 and 8.5, respectively. The action of the OHPAs is considered the link between the initial fermentation stages and the ultimate methanogenic phase.

Table 1.1. Acetogenic, methanogenic, and sulphate-reducing reactions involved in the anaerobic degradation of organic matter.

Reaction Type	ΔG°
Syntrophic	
$CH_3CH_2C00^- + 3H_20 \rightarrow CH_3C00^- + HCO_3^- + H^+ + 3H_2$	+76.1
$CH_3CH_2CH_2C00^- + 2H_20 \rightarrow 2CH_3C00^- + H^+ + 2H_2$	+48.3
$CH_3CH_20H + H_20 \rightarrow CH_3C00^- + H^+ + 2H_2$	+9.6
Methanogenic	
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_20$	-33.9
$CH_3C00^- + H_20 \rightarrow CH_4 + HCO_3^-$	-31.0
Sulphate-reducing	
$CH_3CH_2C00^- + ¾S0_4^{2-} \rightarrow HS^- + 4H_20$	-37.7
$CH_3CH_2CH_2C00^- + ½S0_4^{2-} \rightarrow 2CH_3C00^- + ½HS^- + ½H^+$	-27.8
$CH_3CH_20H + ½S0_4^{2-} \rightarrow CH_3C00^- + ½HS^- + ½H^+ + H_20$	-66.4
$4H2 + S0_4^{2-} + H^+ \rightarrow HS^- + 4H_20$	-38.1
$CH_3C00^- + S0_4^{2-} \rightarrow 2HCO_3^- + HS^-$	-47.6

Source: After Thauer et al. (1993).

The final step in the anaerobic treatment of wastewater is methanogenesis. Methanogens are strictly anaerobic *Archaea* that can be subdivided into two groups: (i) hydrogenophilic or hydrogenotrophic species, which form methane by the reduction of H_2/CO_2 and (ii) acetoclastic or acetotrophic methanogens, which generate methane by acetate decarboxylation (Fig. 1.1a; Table 1.1; Ferry, 1999). Acetoclastic methanogens are considered the more important methanogenic species, as 70% of the total methane generated during AD of domestic sewage is via this pathway (Grotenhuis, 1992; Lettinga, 1995).

1.2.2 Other Microbial Groups involved in AD

1.2.2.1 Sulphate-reducing bacteria (SRB)

In the presence of sulphate, sulphite or thiosulphate, SRB can grow heterotrophically or lithotrophically on a range of different substrate types and are important members of the microflora of a typical anaerobic digester. The SRB are a group of strictly anaerobic bacteria that share the ability to use sulphate as a terminal electron acceptor for the oxidation of molecular hydrogen and a wide variety of organic compounds (O'Flaherty, 1997). Interestingly, it appears that many species of SRB can grow acetogenically in the absence of sulphate, thus explaining the widespread occurrence of these organisms in anaerobic digesters treating wastewaters with low sulphate concentrations, such as domestic sewage (Hao et al., 1996). The problems associated with the anaerobic treatment of sulphate-containing wastewaters result from the ability of the SRB to use a range of substrates in the presence of sulphate and therefore interact competitively with the other bacteria involved in the process, resulting in the formation of H_2S rather than methane (Fig. 1.1a; Colleran et al., 1995; O'Flaherty et al., 1998a; O'Reilly and Colleran, 2004). This competition is favourable to SRB based on thermodynamic considerations (Table 1.1), but the actual outcome under operational conditions is not straightforward. A broader discussion of the SRB, and their role in AD, is provided by O'Flaherty and Colleran (2000). The main manifestations of SRB-related problems include:

1 A reduction of methane yield;

2 The inhibitory effect of H_2S on many bacterial trophic groups involved in AD, thus reducing reactor performance (Widdel, 1988; O'Flaherty et al., 1998b; O'Flaherty and Colleran, 1999);

3 Malodour, corrosion of piping, pumps, etc., necessity for scrubbing of the biogas and effluents to meet discharge standards (Rinzema, 1988).

1.2.2.2 Homoacetogenic and fatty-acid synthesising bacteria

The homoacetogenic bacteria are another trophic group that may play a role in the AD process (Ryan et al., 2004). These organisms are capable of both autotrophic and heterotrophic growth, generating acetate as a sole end product from either H_2/CO_2 or multi-carbon compounds. Although the role of these bacteria is unclear, there is some evidence to suggest that they may be important in some systems (Zhang and Noike, 1994; O'Flaherty et al., 1998a; Kotsyurbenko et al., 2001; Ryan et al., 2004). The most common autotrophic homoacetogenic genera are *Clostridium* and *Acetobacterium*, while the heterotrophic homoacetogens are distinct in that they are unable to grow on hydrogen and carbon dioxide (Dolfing, 1988).

Other bacteria, also found in anaerobic digesters, can act as fatty-acid synthesisers. These organisms, mainly belonging to the genus *Clostridium*, produce lower fatty acids from acetate and/or ethanol when the concentration of hydrogen is high, thereby reversing the reactions of the syntrophic bacteria. This may be an indicator of reactor instability (Smith and McCarty, 1988). The formation of butyrate and caproic acid, from acetate and ethanol, was first investigated by Barker and Taha in 1942. More recently, bacteria that can convert acetate to propionate and act as a hydrogen sink by means of hydrogenases have been isolated (Laanbroek et al., 1982; Schink, 1984). The role of these bacteria is unclear but they are thought to be involved in ethanol degradation and may also act cooperatively with homoacetogenic bacteria in propionate formation from C1 (single carbon) compounds (Goldberg and Cooney, 1981).

1.2.3 Role of as yet Undescribed Microorganisms

The use of culture-independent methods has recently highlighted the role of many other, as yet uncultured, groups of microbes in AD, such as the non-thermophilic crenarchaeota (Collins et al., 2004b, 2005b). However, the exact role of these groups remains unknown, and much research remains to be carried out in this area to elucidate the role and impact of these groups on the AD process (Collins et al., 2005b).

1.2.4 Knowledge Gaps in the Biochemistry of AD

Notwithstanding the large amount of information generated by the microbiological and biochemical studies of methanogenesis or the use of established methods for the assessment of specific methanogenic activity (SMA; Colleran et al., 1992; Soto et al., 1993) and anaerobic biodegradability (Colleran et al., 1992), knowledge of the microbiology and biochemistry of AD remains incomplete. The principal reason is that the technical and scientific basis for the successful isolation and culture of all microorganisms in the methanogenic consortium does not exist. Given the complexity of the methanogenic consortium and the interdependency of its members, it is unlikely that many of these organisms will be independently culturable, while displaying the same metabolic characteristics as in the consortium. However, the application of combined ecology-function techniques to obtain phylogenetically defined data offers a solution which, to some extent, recognises the problems associated with attaining pure cultures, and offers a route to circumvent the necessity for it. This approach is the focus of more recent work on anaerobic consortia, which is now beginning to produce important results (Collins et al., 2005d; McKeown et al., 2009).

1.3 Anaerobic Digestions for Sewage/ Municipal Wastewater Treatment

> 'The greatest challenge in the water and sanitation sector over the next two decades will be the implementation of low cost, hygenic sewage treatment that will at the same time permit selective reuse of treated effluents for agricultural and industrial purposes.' (Looker, 1998)

The currently applied paradigm for municipal wastewater treatment in the EU does not meet basic sustainability criteria, and runs counter to the stated goals of recent EU policies regarding sustainable development. The present system is a disposal-based linear system, with aerobic microbiology as the core technology. This results, in the case of activated sludge plants, in a requirement for large capital investment, heavy usage of fossil fuels, high-technology operational control and the generation of large quantities of sludge requiring treatment before

reuse/recycle (Grommen and Verstraete, 2002). Based on experience from past mistakes in sewage treatment technology, the definition of what is sustainable is now clearer. Although site-specific properties must be taken into account, there are core elements of sustainable treatment that should be fulfilled in each case (Table 1.2).

Table 1.2. Criteria for sustainability in the treatment of wastewater.

1	No dilution of high strength wastes with clean water.
2	Maximum possible recovery and re-use of treated water and by-products (i.e. biogas energy, fertilisation).
3	Application of efficient, robust and reliable treatment/ conversion technologies, which are low cost (in construction, operation, and maintenance), which have a long lifetime and are straightforward with respect to operation and maintenance.
4	Applicable at a wide range of scales.
5	Acceptable to the general population, leading to a high level of self-sufficiency in all respects.

Source: Lettinga, 1995; Grommen and Verstraete, 2002.

Specific examples of how these criteria can be implemented in an Irish context include:

1 Reduction of investment costs:
   - decentralisation;
   - change from gravity sewers to vacuum sewers;
   - increase the volume-time yields of bioreactors.

2 Reduction of operating costs:
   - increase the concentrations of pollutants:
     – rain water separation;
     – reduction of drinking water consumption;
     – addition of kitchen or other locally available organic wastes.

These changes, and indeed the concept of sustainability in wastewater treatment, could be facilitated through a change from *aerobic* to *anaerobic* microbiology as the core method of an advanced technology for environmental protection and resource preservation. This is because anaerobic treatment in high-rate reactors represents, combined with other methods (e.g. nutrient removal and hygenisation), a sustainable and appropriate wastewater treatment system for lower-strength wastewaters, such as sewage (Lettinga et al., 2001; McHugh et al., 2003a; Table 1.3). A further major advantage of the AD approach is that it can be applied to either very small or very large scales, which makes AD a sustainable option for a growing community.

Table 1.3. Advantages of anaerobic digestion treatment.

1	Very low energy demand.
2	Production of valuable energy in the form of methane.
3	Low investment costs and low space requirement.
4	Applicable at small as well as large scale.
5	Low production of excess sludge, which is well stabilised.
6	Low nitrogen and phosphorus requirements.
7	High loading capacity (5–10 times that of aerobic treatment).
8	High treatment efficiencies.
9	Suitable for areas where there are long-term periods without discharge of wastewater.
10	Effluents contain valuable fertilisers (ammonium salts).

The advantages of the anaerobic microbiological approach have been recognised for many years. In the 1980s, Jewell (1985) noted that 'there is little doubt that development of a cost-effective and efficient anaerobic sewage treatment alternative [to conventional activated sludge systems] would be one of the most significant advances in waste treatment'. Lettinga et al. (1987) fully agreed, commenting that 'a satisfactory application to raw domestic sewage would represent the maximum possible accomplishment for high-rate anaerobic treatment systems'. In fact, since the 1990s, high-rate anaerobic treatment of municipal streams has been applied, although to date only in regions with a warm climate (e.g. Colombia, Brazil, and India), replacing the much more costly activated sludge processes of diminishing the required pond areas (van Haandel and Lettinga, 1994; Foresti, 2002; Aiyuk and Verstraete, 2004). Thus, despite widespread historical scepticism related to their applicability for low-strength wastewaters, more efficient anaerobic systems have been developed, and they are being successfully applied for the treatment of low-strength discharges, such as domestic wastewater (reviews: Segezzho et al., 1998; Mahmoud et al., 2003). The challenge now is to produce innovative technological solutions to bring the benefits of AD for sewage treatment to regions with a colder climate, such as Ireland and many other EU member states (Lettinga et al., 2001; Collins et al., 2005a).

In summary, although anaerobic biofilm reactor technology is a sustainable, highly advantageous approach for municipal and industrial wastewater treatment, knowledge gaps exist, which, if addressed, would open up the possibility of incorporating AD as the core technology for municipal wastewater treatment in countries with a temperate climate, thereby realising a major commercial and technological opportunity and allowing for the sustainable growth of communities.

1.4 Biotic and Abiotic Factors Influencing the Microbiology of Anaerobic Domestic Sewage Treatment

A number of factors will affect the operation of the high-rate AD of domestic sewage, and indeed whether this approach will find widespread acceptance in regions with temperate, as well as tropical climates (Mahmoud, 2002; Aiyuk et al., 2005). These factors include the required effluent quality, the ambient temperature, the volumetric loading rate, wastewater constituents, available land and economic issues (availability of equipment and technical support, etc.). Many of these potential drawbacks can be overcome for regions with a warm climate, by the selection of appropriate pre- and post-treatments (Aiyuk et al., 2005). The underlying microbiological stability and efficiency of the process, however, is also crucial for the successful implementation and expanded application of AD technology. In addition to the operational parameters applied, a number of biotic and abiotic factors have been shown to affect the microbial consortia and the formation of well-settling sludge during AD of wastewater, including temperature, wastewater constituents, pH, the presence of essential nutrients and the absence of excessive concentrations of toxic compounds in the influent (Grotenhuis, 1992; Seghezzo, 2001; Aiyuk et al., 2005). In the case of domestic wastewater, nutrients are abundantly available, and an adequate and stable pH is set by the presence of the carbonic system.

1.4.1 Granulation during Anaerobic Treatment of Domestic Sewage

The UASB technology, which is applied in many countries to treat domestic sewage, (e.g. Brazil, Columbia, Mexico, Uruguay; van Haandel and Lettinga, 1994), relies on the efficient retention of an active biomass and adequate biomass/wastewater contact to operate effectively (Aiyuk et al., 2005). This is also a requirement for derivative systems, such as expanded granular sludge bed (EGSB) or IC reactors. The retention of biomass in such high-rate systems often relies on the formation of well-settling, granular biomass. A range of factors has been identified as influencing granulation – including the selection pressure effect (where suspended biomass is washed out of the system) (Hulshoff Pol, 1989), low surface tension (Thaveesri et al., 1995), the presence of inorganic nuclei (Hulshoff Pol et al., 1983; Alibhai and Forster, 1986) and the presence of readily acidifiable COD (RACOD; Vanderhaegen et al., 1992), which encourage extra-cellular polymer formation by the microbes. The presence of extra-cellular polymeric substances (EPS) is an important factor in determining the strength of granules and the likelihood of granule loss due to gas bubble flotation (de Beer et al., 1996; O'Flaherty et al., 1997).

From the microbiological point of view, a general consensus is apparent in the literature that the acetotrophic methanogen, *Methanosaeta* sp., plays a key role in granulation, where the clumps formed by the growth of these filamentous microorganisms function as nucleation centres that initiate granule development (MacLeod et al., 1990; Hulshoff Pol et al., 2004), followed by subsequent colonisation by acetogenic bacteria and hydrogenotrophic methanogens, which often leads to the layered granular biofilm structure reported by several authors (Vanderhaegen et al., 1992; Sekiguchi et al., 1999; Collins et al., 2005b).

There are conflicting reports as to whether granulation can be supported during the treatment of domestic sewage in UASB reactors. In principal, given the low concentration of RACOD present in domestic sewage and the fact that the temperature is normally not optimal for growth of *Methanosaeta* sp., granulation may seem unlikely, and van Haandel and Lettinga (1994) have reported no granulation in any of the UASB reactors then treating domestic sewage at full scale. Indeed, the loss of granular sludge integrity has been reported to cause problems of increased sludge washout and decreased reactor efficiency during AD of domestic sewage and synthetic analogues (Grotenhuis, 1992; Aiyuk and Verstraete, 2004). Granule disintegration has been suggested to result from the low substrate concentration of domestic sewage (Grotenhuis, 1992)

and from mass transfer limitations caused by low upflow velocities and low biogas production. The latter two problems cause the SMA of the reactor sludge to decrease, which in turn leads to internal mass transfer limitation and the accumulation of inert organic material at the centre of granules and ultimately granule disintegration (Grotenhuis, 1992).

Despite these issues, Foresti (2002), and others, reported that granulation occurred during treatment of both raw and settled domestic sewage (e.g. Barbosa and Sant'Anna, 1989; Seghezzo et al., 2001). Furthermore, reports have indicated that granulation proceeds well when EGSB reactors are employed for domestic sewage treatment (Kalogo and Verstreate, 2001). This suggests that the formation of granules on domestic sewage may be heavily influenced by the applied upflow velocity. Furthermore, research has also demonstrated that the process may be enhanced by adding flocculating agents (Kalogo and Verstreate, 2001).

What is clear from operational experience is that granulation is not, in fact, a prerequisite for the successful UASB treatment of domestic sewage (van Haandel and Lettinga, 1994). However, for enhanced efficiency of the AD process and for its application for domestic sewage treatment to regions with lower ambient temperatures, a pre-treatment step to remove suspended solids is a likely scenario (Kalogo and Verstraete, 2001; Seghezzo et al., 2001; Foresti, 2002; Aiyuk et al., 2005). This leaves open the possibility of a high-rate, low hydraulic retention time (HRT) treatment of clarified sewage in EGSB/UASB reactors, optimised by pre- and post-treatment steps, even in regions with temperate climates. The implementation of AD in these regions will be greatly aided by a systematic knowledge of the biotic and abiotic factors influencing granulation during domestic sewage treatment at specified operating conditions. From a microbiological point of view, there is no doubt that fully elucidating the requirements and conditions required for anaerobic sludge granulation, using the range of modern microbiological and analytical techniques now available, and discussed above, would be a significant step forward and would enhance the operational efficacy and attractiveness of AD for domestic sewage treatment.

1.4.2 Role of Soluble Wastewater Constituents on the Microbiology of AD

The composition and anaerobic biodegradability of sewage are variable, and dependent on location (Henze and Leddin, 2001; Seghezzo, 2001; Mahmoud, 2002). Depending on the temperature and level of fractionation, the anaerobic biodegrability of raw sewage ranges from 64% to 79% (Elmitwalli, 2000; Seghezzo, 2001; Mgana, 2003). There may also be a significant number of toxic compounds present in sewage, including, for example, organic solvents, phenols, halogenated aromatics, alkylphenols and azo-dyes. The presence of such toxicants will be dependent on location and whether particular industrial wastewaters are discharged into municipal sewers. While shock loadings of such compounds will undoubtedly cause problems for any biological treatment process, the anaerobic treatment approach has been shown to be quite robust with respect to exposure to toxicants (Colleran et al., 1992). Indeed, AD has been employed successfully for the treatment of wastewaters containing phenols (Collins et al., 2005a), chlorinated phenols (Collins et al., 2005c) and organic solvents such as acetone (Enright et al., 2005) at high rate, and at low-ambient temperatures. In addition, successful mesophilic treatment has been reported for wastewater containing a wide variety of toxic compounds (Henry et al., 1996; Mahony et al., 2001).

1.4.3 Influence of Operation under Sub-mesophilic and Psychrophilic Temperature Conditions on Methanogenesis and AD of Domestic Sewage

The operation of high-rate AD under the low-ambient temperatures typical of domestic sewage was for many years considered unfeasible (Rebac, 1998). Moreover, due to the low-strength nature of domestic wastewater, with consequent low biogas yield, heating anaerobic digesters to the usual mesophilic (30–37°C) temperatures may not be economically attractive for countries with temperate or cool climates. However, research has shown that, with appropriate reactor design and operation, successful low-temperature reactor operation is feasible (Lettinga et al., 1999, 2001; Rebac et al., 1999; Collins et al., 2004a). The application base of AD has consequently broadened. Recent advances in anaerobic bioreactor design have overcome the limitations associated with applying AD

at reduced temperatures (low-temperature anaerobic digestion [LTAD]). The treatment of low-strength wastewaters at low temperature has benefited from the use of the EGSB bioreactor (Rebac et al., 1999, Kato et al., 1999, Chu et al., 2005), the design of which offers the benefit of greater mixing intensities than alternative reactor designs, resulting in greater contact between the wastewater and biomass through increased height to diameter ratio and effluent recirculation (Puyol et al., 2009). Such approaches have led to the successful LTAD of a wide variety of wastewaters (McKeown et al., 2009).

Methanogenesis at lower temperatures has been described in a variety of environments, including tundra and permafrost soils, pond sediments and those of deep-lake ecosystems (Conrad et al., 1989; Conrad and Wetter, 1990; Kotsyurbenko et al., 1996; Franzmann et al., 1997; Schulz et al., 1997; Nozhevnikova et al., 2000). In these environments, AD of endogenous organic matter is performed by numerous hydrolytic, fermentative, acetogenic, syntrophic and methanogenic microorganisms; and low temperature methanogenesis in natural habitats is considered a significant contributor, in terms of global methane emissions to the atmosphere (Rasmussen and Khalil, 1981). Furthermore, several species of psychrophilic acetogenic bacteria have been described, all of which belong to the genus *Acetobacterium* sp., and include *A. carbinoculum* (Conrad et al., 1989), isolated from lake sediment and *A. bakii, A. paludosum* and *A. fimetarium* (Kotsyurbenko et al., 1995), which were isolated from the silt of a polluted pond, a boreal fen, and cattle manure digested at low temperature, respectively. Two species of psychrophilic methanogens, *Methanococcoides burtonii* and *Methanogenium frigidum*, have been described by Franzmann et al. (1992, 1997), and evidence has been obtained for psychrophilic methanogenic communities in lake sediments and peat bogs (Nozhevnikova et al., 2001).

Propionate degradation has been described as the rate-limiting step in psychrophilic methanogenesis (Rebac, 1998). Despite this, propionate-degrading consortia could be enriched at 10°C, and significant propionate degradation and methane formation was measured at 5°C during laboratory and pilot-scale reactor trials (Lettinga et al., 1999). Furthermore, although most reactions in the biodegradation of organic matter require more energy to proceed at low temperatures than 37°C, some reactions, such as hydrogenotrophic sulfate reduction, hydrogenotrophic methane production and acetate formation from hydrogen and bicarbonate, require less energy (Lettinga et al., 2001).

A strong temperature effect on the maximum substrate-utilisation rates of microorganisms has been observed by many researchers (Lawrence and McCarty, 1969; Huser et al., 1982; Westermann and Ahring, 1987; Wu et al., 1993; Kettunen and Rintala, 1997). Lowering the operational temperature of continuously stirred tank reactors (CSTR) has been reported to lead to a decrease in the maximum specific growth and substrate-utilisation rates, but may also result in an increased net biomass yield of the methanogenic population (Lin et al., 1987) or the acidogenic sludge (van Lier et al., 1997). Temperature effects on substrate utilisation have been described mathematically by using, for example, the Arrhenius equation (Pavlostathis and Giraldo Gomez, 1991). However, it is difficult to describe with one single equation the changes in kinetic parameters induced by temperature for the various organisms present in anaerobic consortia.

To date, although satisfactory reactor performance and the development of the methanogenic activity of psychrophilically grown granular sludges has been discussed for the treatment of a wide range of wastewaters (Table 1.4; Banik et al., 1997; van Lier et al., 1997; Rebac et al., 1999; Collins et al., 2003, 2004a; McHugh et al., 2003a, 2004; Kearney et al., 2004), the prevalence of psychrophilic microorganisms in anaerobic sludge has not yet been demonstrated conclusively. Rather, mesophiles have been shown to grow under low-temperature conditions and exhibit psychrotolerant tendencies, during which time the specific methanogenic activities of these consortia, when tested within the mesophilic range (25–37°C), increased dramatically under psychrophilic conditions (Banik et al., 1997; Rebac et al., 1999; Collins et al., 2003; McHugh et al., 2003a, 2004, 2005a; McKeown et al., 2009). However, psychrophilic bacteria and archaea are very likely to be present in anaerobic digesters, albeit in insufficient numbers, relative to psychrotolerant mesophiles, to be able to manifest in the temperature-activity profiles used in these studies (Lettinga et al., 2001). Generally, reactor trials of longer duration (>1000 days) may be required to cultivate organisms with activity optima within the psychrophilic range. However, SMA assays carried

out by Collins et al. (2005c) and McKeown et al. (2009) suggested the emergence of psychrophilic butyrate- and propionate-degrading bacteria, and hydrogenotrophic methanogens, in granules from EGSB reactors during long-term trials (250–600 days), which were seeded with previously psychrophilically grown sludges.

1.4.4 Role of Suspended Solids and Hydrolysis in AD of Domestic Sewage

In addition to the general microbiology of AD at low temperatures, an additional temperature-mediated effect, with respect to the treatment of domestic sewage, will be the role of hydrolysis, which is the rate-limiting step in the AD process (Pavlostathis and Giraldo-Gomez, 1991; O'Flaherty et al., 2010). During the treatment of domestic sewage, the hydrolysis of suspended solids proceeds first by conversion mechanisms, such as cell lysis, non-enzymatic decay, phase separation and physical breakdown, followed by chemical hydrolysis (Seghezzo, 2001; Batstone et al., 2004). The removal of suspended solids during AD of sewage occurs by physical processes – such as settling, adsorption and entrapment first – followed by hydrolysis and methanogenesis, processes which depend principally on temperature and on solids retention time (SRT; Zeeman and Lettinga, 1999; Seghezzo, 2001). The lower the temperature, the longer the SRT required in a one-step UASB process to degrade the solids (Zeeman et al., 2001). Indeed, if the ambient temperature is low, the pre-removal of suspended solids may be necessary to allow for the operation of high-rate AD at low HRTs and to avoid problems such as the deterioration of sludge quality, floatation and poor treatment efficiency (Elmitwalli, 2000; Seghezzo, 2001). A number of process configurations have been devised to address these issues, and these have expanded the temperature range for the successful AD of domestic sewage (Seghezzo et al., 1998, 2002; Aiyuk and Verstraete, 2004, 2005).

1.5 Key Knowledge Gaps associated with Anaerobic Digestion Technology addressed by this Project

1.5.1 Historical Requirement for Heating of Anaerobic Bioreactors

The application of AD technology to the direct treatment of municipal and low-strength industrial wastewaters has been delayed by a number of knowledge gaps. First, anaerobic granular bioreactors are normally heated (37–55˚C) in order to achieve high rates of microbial activity. This can consume 20–30% of the produced biogas energy and has a significant impact on process economics. However, municipal wastewaters, and those from a broad range of industries in temperate climates, are discharged at temperatures well below 20˚C. *This requirement has severely limited the application of AD biofilm technology to all high-volume, low-strength influents because of high heating costs and relatively low biogas yields (arising because of the low organic matter concentration in the influent)*. For this reason, the major application of high-rate AD has been to high-strength industrial wastewaters (e.g. from food processing, brewing, etc.), where high-rate AD has been tremendously successful (McHugh et al., 2003a).

Anaerobic digestion for municipal or domestic wastewaters, in temperate climates, is applied only to the settled organic solids fraction (primary and secondary sludge) for CSTRs (Metcalf and Eddy, 1991). This is extremely beneficial and can provide much of the energy requirements of municipal treatment plants. However, if unheated or psychrophilic AD could also be applied to the *direct treatment* of municipal streams, major energy, economic and environmental benefits could be achieved (Zeeman and Lettinga, 1999; Elmitwalli, 2000; Zeeman et al., 2001; Seghezzo, 2001).

As temperature strongly affects the rates of anaerobic conversion processes, improvements must be made to conventional high-rate reactor design to allow operation at sub-optimal temperatures and also to treat low-strength wastewaters. The research group has carried out extensive basic research at laboratory scale – over a 3–5-year period, using a wide range of synthetic wastewaters – to address this topic (e.g. McHugh et al., 2003b; Collins et al., 2005a and b; McKeown et al., 2009; Table 1.4) and has also treated successfully, under high-rate conditions, a variety of 'real' industrial wastewaters at low temperatures (McHugh et al., 2003b; Kearney et al., 2004). The results of the trial indicated that high-rate AD technology could be applied successfully, at temperatures ranging from 10–20˚C, to medium- and high-strength wastewaters, results that have been supported by the findings of leading international groups (Rebac et al., 1999; Lettinga et al., 2001; 2005).

These results provided a clear justification for comprehensive trials to investigate the potential application of low-temperature AD for municipal wastewater treatment in temperate climates.

1.5.2 Reactor Configuration

Municipal wastewater, or sewage, comprises: (i) domestic wastewater, generated from bathrooms and toilets, and activities such as washing and cooking, etc.; (ii) industrial wastewater, from industries using the same sewage system for their effluents (treated, partially treated or untreated); and (iii) rain water, particularly in the case of sewer systems constructed for both wastewater and storm water. Most of the reported results for AD of municipal wastewaters refer to systems operated with operational temperatures higher than 20˚C and employing the UASB reactor with a secondary settler as the main process configuration (Behling et al., 1997; McHugh et al., 2003a). In general, sewage (average 200 mg COD l^{-1}) was treated efficiently (75–95% COD removal) at HRTs as low as 2 h, without problems of operational stability (Seghezzo et al., 1998; Kalogo and Verstraete, 1999; Leitão et al., 2005).

There is much less data available for AD of municipal wastewaters under temperature conditions closer to those found in Ireland (an issue that was addressed by this project). Wang (1994), proposed the two-stage HUSB (hydrolysis upflow sludge bed reactor)-UASB, with secondary settlers, for treatment of sewage at temperatures below 20˚C, as under these conditions hydrolysis of suspended solids proceeds slowly and they can accumulate, decreasing the sludge retention time and preventing the development of the methanogenic *Archaea* in the reactor (Cavalcanti et al., 1999; Mahmoud et al., 2003).

Readily acidifiable chemical oxygen demand (RACOD) has been prescribed as important in influencing the build-up and maintenance in an UASB reactor of granular sludge (Vanderhaegen et al., 1992). Readily acidifiable chemical oxygen demand is low in domestic sewage, with only 25–50 mg l^{-1} on a total COD concentration of 500 mg l^{-1}, making granulation unlikely. Indeed, most tropical UASB reactors are operated using a flocculant sludge type (Leitão et al., 2005). This does limit the loading rate applicable to the reactors, and may result in occasional operational instability (van Haandel and Lettinga, 1994). However, the EGSB reactor concept does allow granulation on domestic sewage, as the recirculation of influent wastewater supplies the necessary hydrodynamic force for granule formation (Kato, 1994). Furthermore, low-strength sewage wastewater may lead to increasing starvation conditions

Table 1.4. Particulars of reactor, wastewater and inoculum type, operating conditions and efficiency, and trial duration for the laboratory-scale psychrophilic reactors R1–R12.

Reactor type [a]/ Parameter	1/AF	2/E	3/ HEA	4/ HEA	5/ HEA	6/ HEA	7/ HEA	8/ HEA	9/ HEA	10/ HEA	11/ HEA	12/ HEA	13/ HEA
Wastewater[b]	VFA[c] High	VFA high	VFA medium	VFA medium	Sucrose high	VFA high	Whey low	Whey high	VFA medium	Phenol medium	VFA medium	TCP[d] medium	Solvents medium
HRT[e]	48 h	48 h	8 h	8 h	12 h	12 h	18 h	18 h	24 h	24 h	48 h	48 h	4h
Operating Temperature °C	18	18	18	18	16	16	12	12	18	18	15	15	11
OLR[f] kg COD m^{-3} d^{-1}	5	5	15	15	20	20	0.5–1.5	5– 15	5	5	2.5	2.5	5
Inoculum[g]	G	S	G	G	G	G	G	G	G	G	G	G	G
Mean COD Removal (%)	76	72	94	95	65	90	88	95	96	95	97	89	90
Average CH_4 in biogas (%)	67	64	67	69	61	66	63	68	72	68	73	67	75
Trial duration (d)	90	90	250	250	250	250	500	500	415	415	370	370	625

a: AF = Anaerobic Filter, E = EGSB, HEA = hybrid EGSB-Anaerobic Filter; b: high = 10, medium = 5, low = 1 kg COD m-3; c: Volatile fatty acids; d: 2,4,6- Trichlorophenol; e: HRT = hydraulic retention time; f: OLR = Organic Loading Rate; f: g = Anaerobic granular sludge, S = suspended/flocculant biomass.

Source: Condensed from Collins et al., 2005a, b and Enright et al., 2005 (reactor number 13).

in a UASB reactor sludge bed, and, coupled to low loading rates (especially if the inoculum is acquired from a full-scale plant with a high organic loading rate [OLR]), may mean that granules start to decay from their centres in a UASB due to an acute mass transfer limitation of soluble substrate and thus become buoyant and wash out from the system. However, in an EGSB, the applied shear stress and erosion from the hydrodynamics of the in-reactor liquid can disintegrate decaying/buoyant granules into finer particles, and thus achieve a higher surface/volume ratio, while also providing nuclei for new granule formation (Kalogo and Verstraete, 1999).

The O'Flaherty group at the NUI, Galway has developed anaerobic bioreactors based on an EGSB/anaerobic filter (EGSB/AF) hybrid design, specifically for low-temperature operation and has demonstrated their effectiveness for a number of strength wastewaters (e.g. Collins et al., 2003; 2005a and b; McHugh et al., 2004, 2005a and b; Connaughton et al., 2005). Adding the AF moiety enhances performance in two ways: (i) an effluent polishing step is in place to improve treatment efficiency and buffer the effect of shock hydraulic or organic loadings; and (ii) suspended solids are removed efficiently by the filter section (Connaughton et al., 2005). When applied at low temperatures, this configuration appears to offer the potential to advance the state-of-the-art with respect to the anaerobic treatment of municipal wastewaters.

Table 1.5. Performance of a modular anaerobic membrane reactor during treatment of synthetic wastewaters in preliminary trials.

COD (mg/L)	Suspended solids (mg/L)	Trial duration (d)	% COD removal (mean)	% SS removal (mean)
300	40	25	81	85
600	80	28	90	97
900	120	41	92	99

Finally, innovative reactor designs based on membrane biofilm technology appear very promising for low-strength wastewater applications; they have been the focus of much recent research (Hu and Stuckey, 2006). The applicants have designed a modular membrane-based reactor and carried out a preliminary trial using synthetic domestic wastewater – with highly encouraging results (Table 1.5).

1.6 Rationale for this Project

This project was justified based on sound preliminary Irish and international research. Salient facts include:

1 Anaerobic digestion wastewater treatment technology is a well-established and highly successful industrial wastewater treatment (Lettinga, 1995; Lettinga et al., 2001; Grommen and Verstraete, 2002; McHugh et al., 2003a).

2 Anaerobic digestion has been implemented successfully for treating municipal wastewaters in sub-tropical regions (average temperature >20˚C) for several years, with very positive performance and stability data (Leitão et al., 2005 and references therein).

3 High-rate, low-temperature anaerobic treatment of a wide variety of wastewaters has been demonstrated at laboratory-scale, with similar efficiency and loading rates to mesophilic trials (Table 1.4; McHugh et al., 2003a, b, c; Collins et al., 2004, 2005; McKeown et al., 2009). In addition, preliminary trials with low-strength wastewaters comparable to municipal or low-strength industrial streams in terms of COD strength were very positive (Tables 1.4 and 1.5; Connaughton et al., 2005).

4 Anaerobic consortia enriched from reactor sludge are capable of mineralising key anaerobic substrates, such as acetate, propionate and butyrate at temperatures as low as 4˚C efficiently (Enright et al., 2005);

5 It is possible to evaluate the long-term microbial community dynamics, activity and biofilm structure in anaerobic bioreactors, and this information underpins the design and successful operation of anaerobic bioreactors (McHugh et al., 2003b; Collins et al., 2003; McHugh et al., 2005b; McKeown et al., 2009).

Given this background of research and full-scale application, and the clear advantages of AD, when compared to the aerobic approach, the research group formed the hypothesis that:

Direct anaerobic treatment of municipal or low-strength industrial wastewaters under Irish conditions is both feasible and desirable.

1.7 Aims and Scope of the Project

In order to test the research hypothesis, a number of laboratory-scale bioreactor trials were carried out, the results of which form the basis for this report. Section 2 describes a comparative long-term trial where three laboratory-scale EGSB/AF hybrid bioreactors were used to treat a synthetic sewage at low (250 mg $CCOD^{-1}$); medium (500 mg COD l^{-1}) and high (750 mg COD l^{-1}) concentrations at 37˚C, 15˚C and 10˚C. Section 2 outlines both reactor performance and biomass characteristics underpinning performance. Section 3 describes the investigation of the performance of a novel bioreactor during a long-term trial treating synthetic sewage (500 mg COD l^{-1}) at 12˚C. Section 4 discusses the implementation of laboratory-scale LTAD for the treatment of raw and primary settled sewage from the Mutton Island treatment plant. Section 5 summarises the main conclusions arising from this work and recommends the next actions with respect to the implementation of low-temperature anaerobic sewage treatment.

2 High-rate Anaerobic Digestion of Synthetic Sewage in Anaerobic Hybrid Bioreactors at 10°C, 15°C and 37°C

This section presents the results of an investigation as to the feasibility for direct anaerobic treatment of sewage using laboratory-scale EGSB/AF hybrid bioreactors. This reactor configuration was chosen for the initial trials as it had been successfully employed for LTAD of a variety of industrial wastewaters previously (McHugh et al., 2003a, b, c; Collins et al., 2004, 2005; McKeown et al., 2009). A chemically defined, synthetic sewage (SYNTHES; Aiyuk and Verstraete, 2004) was employed for these initial experiments to allow a well-controlled evaluation of system performance and the characteristics of bioreactor biomass under low temperature operating conditions.

The principal hypothesis being tested was that long-term, low-temperature (15°C and 10°C) anaerobic treatment of synthetic sewage could be achieved successfully, with operational performance comparable to that of bioreactors operating in the conventional, mesophilic temperature range (37°C) and that sucessful development of methanogenic microbial communities can be achieved. The ability of a mesophilic seed sludge inoculum to adapt to low-temperature conditions, the start-up times achievable and the potential role for an AF section in such bioreactor systems was also investigated. Elmitwalli et al. (1999, 2001) had previously highlighted the importance of filter media in the anaerobic treatment of complex waste streams, such as domestic sewage, with respect to the removal of the various COD fractions, and suggested that a hybrid granular sludge and AF system would be advantageous for such applications.

An additional, and novel, feature of the work presented in this report is the use of molecular tools to describe the methanogenic community structure of biomass in the reactor. This is important for practical applications, as the methanogenic *Archaea* (methanogens) have a very limited substrate range and utilise one 2-Carbon compound (acetate) and several 1–Carbon compounds (e.g. methanol, CO_2 and CO; Ferry, 1999). Indeed, only two genera of the order Methanosarcinales (*Methanosaeta* and *Methanosarcina*) have been shown to use acetate. Despite this, acetate is a key substrate in the AD process, with *c.* 70% of methane in biogas produced via acetate decarboxylation in stable bioreactors (Lettinga et al., 2001). In order for the long-term and successful operation of LTAD, therefore, key methanogenic populations – particularly those capable of using acetate – must be retained in the reactor biomass. The purpose of the molecular biological investigation of the reactor biomass is to determine the stability and identity of the methanogenic and bacterial communities present to evaluate the likelihood of successful long-term process stability.

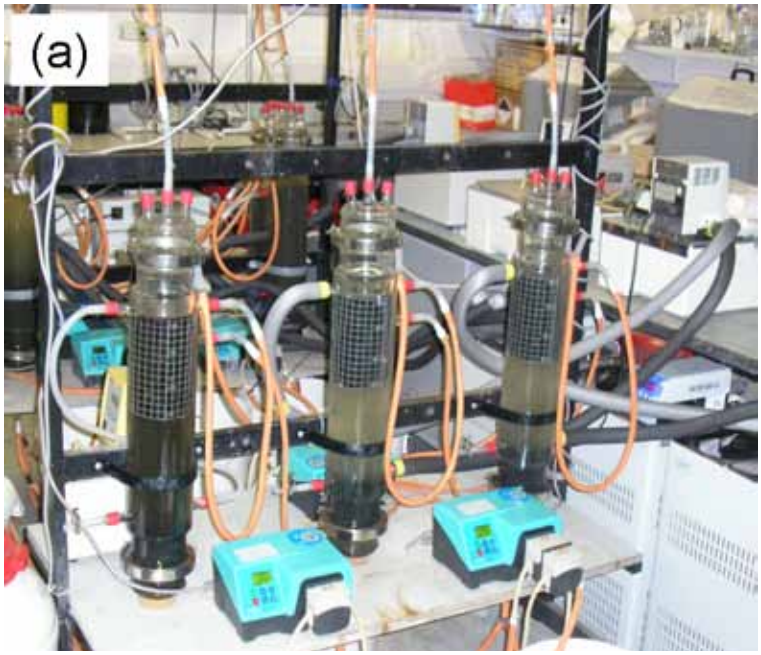
2.1 Experimental

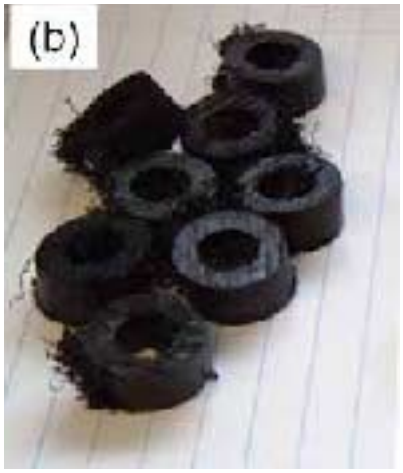
2.1.1 Source of Biomass

Anaerobic sludge granules were obtained from a full-scale, mesophilic (37°C), IC bioreactor at Carberry Milk Products (Ballineen, Co. Cork, Ireland) used to treat dairy production process wastewaters. The volatile suspended solids (VSS) concentration of the granules was 102.8 g VSS l^{-1}.

2.1.2 Bioreactor Design and Operation

Three glass, laboratory-scale (3.5 l; active liquid volume, 3.0 l) EGSB-AF bioreactors, R1–3, were used in the present study (Fig. 2.1a). The upper, 'fixed-film' AF section comprised polyethylene rings (Ø, *c.* 25 mm) randomly packed in a cylindrical, wire-mesh cage (Fig. 2.1a). R1 was inoculated with 11 g VSS $l_{reactor}^{-1}$ of the sludge granules and was operated at 37°C (control). R2 and R3 were each inoculated with 20 g VSS $l_{reactor}^{-1}$ and were operated at 15°C and 10°C, respectively. R1–3 were employed to treat a synthetic sewage wastewater (SYNTHES; Aiyuk and Verstraete, 2004) over three distinct operational phases (Phases 1–3), which were characterised by the strength of the influent: 750 mg chemical oxygen demand (COD) $l_{influent}^{-1}$ in Phase 1; 250 mg COD l^{-1} in Phase 2; and 750 mg COD l^{-1} in Phase 3. The trial was divided into 11 operating periods (PI–XI), which were characterised by changes in HRT and consequent OLR (Table 2.1). Effluent was recirculated through the respective bioreactors to give an applied liquid upflow velocity of 5 m h^{-1} (Phases 1 and 2) or





according to the HACH molybdovanadate method with acid persulfate digestion.

Figure 2.1. (a) Bioreactors R1–R3; (b) anaerobic filter (AF) polyethylene packing rings and (c) biofilm-coated rings sampled from bioreactor R3, at the conclusion of the trial.

2.5 m h^{-1} (Phase 3).

2.1.3 Methanogenic Activity

The SMA profiles of the seed biomass (day 0), and of biomass from R1–R3 on days 405 and 544, were determined at 10, 15 and 37°C. Additionally, R1–R3 biomass samples from days 124, 201, 230, 285, 325 and 371 of operation were tested at the operating temperature of the respective parent bioreactors. Fixed-biofilm samples, recovered from the AF section of R1–R3 at the conclusion of the trial (day 544), were also tested at the respective operating temperature of the parent bioreactors. The SMA of biomass samples was separately assayed against acetate (30 mM), butyrate (15 mM), propionate (30 mM), ethanol (30 mM) and H_2/CO_2 (80:20, v/v) as previously described by Collins et al. (2003). Briefly, biomass samples were washed in 1X PBS and were transferred to sterile 20 ml (soluble substrates) or 60 ml (gaseous substrates) hypovials to achieve a final biomass concentration of 2–5 g VSS l^{-1} in a total volume of 10 ml anaerobic activity test medium (Colleran et al., 1992; Coates et al., 1996). Vials without substrate, or with only pressurised N_2/CO_2 headspace, were used as controls.

2.1.4 Molecular Microbial Community Analysis

Total genomic DNA was extracted from seed sludge and biomass obtained from the reactors during the operation. All biomass samples were mechanically disrupted by manual grinding with a pestle and mortar and diluted 10-fold with deionised and distilled water (DDW). Cells from each 1 ml sample were harvested by centrifugation at 13,000 rpm for 5 min, followed by decantation of the supernatant. The residual pellet was washed with 1 ml of DDW, and centrifuged again in the same manner to ensure a maximal removal of

residual medium. After repeating the washing step 2 times, pellet was resuspended in 1 ml of DDW. Total DNA in the suspension was extracted using an automated nucleic acid extractor (Magtration System 6GC, PSS, Chiba, Japan). Purified DNA was eluted with 100 µl of Tris–HCl buffer (pH 8.0) and stored at -20˚C for further analyses. DNA extraction was performed in duplicate.

Archaeal and bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR) using ARC 787F and ARC 1059R (Takai and Horikoshi, 2000) and Bac8F and Prk1492R (Lane et al., 1991), respectively. Touchdown PCR was conducted using thermal cycler (G-Storm) and the following protocol was applied: initial denaturation at 94˚C for 10 min; 20 cycles of denaturation at 94˚C for 1 min, annealing at 65˚C to 55˚C (reducing by 0.5˚C per cycle) and elongation at 72˚C for 1 min; followed by 20 cycles at 94˚C for 1 min, 55˚C for 1 min, 72˚C for 1 min and final elongation at 72˚C for 30 min (Janse et al., 2004). The PCR products were gel-purified and cloned into pGEM-T Easy vector (Promega, Madison, WI). The cloned gene fragments were sequenced using T7 primer-based PCR and compared against the Ribosomal Database Project (RDP) database. Sequencing alignment and phylogenetic analysis were performed using MEGA 4 software (Tamura et al., 2007).

2.1.5 Analytical Techniques

Samples of R1–R3 effluent were taken thrice weekly to determine total COD (COD_{tot}) and soluble COD (COD_{sol}), and pH according to standard methods (American Public Health Association [APHA], 1998). Biogas production rates were recorded using wet-gas meters, as previously described by Connaughton et al. (2005). Biogas CH_4 concentrations were monitored according to standard methods (APHA, 1998). Acetic acid concentrations were determined by GC-MS (Varian, USA). Phosphate (PO_4^{3-}) was detected using a colorimetric spectrophotometer (HACH DR4000),

2.2 Results and Discussion

2.2.1 Bioreactor Performance: Phase 1

A short start-up was observed for each of the bioreactors at the applied OLR of 1.5 kg COD m^{-3} d^{-1} (PI), with COD_{tot} and COD_{sol}, removal efficiencies of *c.* 70% achieved by day 13 (Fig. 2.2a, b). This performance continued throughout PI, but R2 (15°C) routinely outperformed both R1 and R3. Biogas yields for PI of 300–390 ml d^{-1} were comparable from all three bioreactors, with highest production observed in R2. PII was characterised by a decrease in the HRT (with a consequent increase in OLR to 3 kg COD m^{-3} d^{-1}), which resulted in some biomass washout from each bioreactor. This was associated with a gradual decrease in COD removal efficiencies over a 10-day period, followed by a sustained period (days 138–170) of poor performance in R1–R3, with mean COD_{tot} removal of 44% (Fig. 2.2a). Improved performance was exhibited for R1–R3 for the remainder of PII, with COD_{tot} and COD_{sol} removal of >70%, >50% respectively (Figs 2.2a and b). Biogas quality for this period averaged >71% for R1 and R2 and 63% for R3 (Fig. 2.3a). However, these figures are somewhat misleading as gas chromatography measurements were unavailable during days 125–159, a time of poor performance for R1–R3 (Fig. 2.3b; Table 2.1). The introduction of PIII had no adverse effect on bioreactor performance, with removal efficiencies R1–R3 demonstrating a gradual improvement throughout the period. The final period (PIV) of Phase 1 involved a return to the initial (PI) operational conditions. Performance efficiency remained stable from that observed at the completion of the previous period. Biogas analyses carried out throughout Phase 1 displayed comparable results for R1 and R2, with R3 showing slightly reduced biogas quality (Fig. 2.3a, Table 2.1). Acetic acid concentrations remained very low throughout Phase 1 (0–0.19 mM ml^{-1}), with mean concentrations of just 0.09 mM ml^{-1} (Fig. 2.4a).

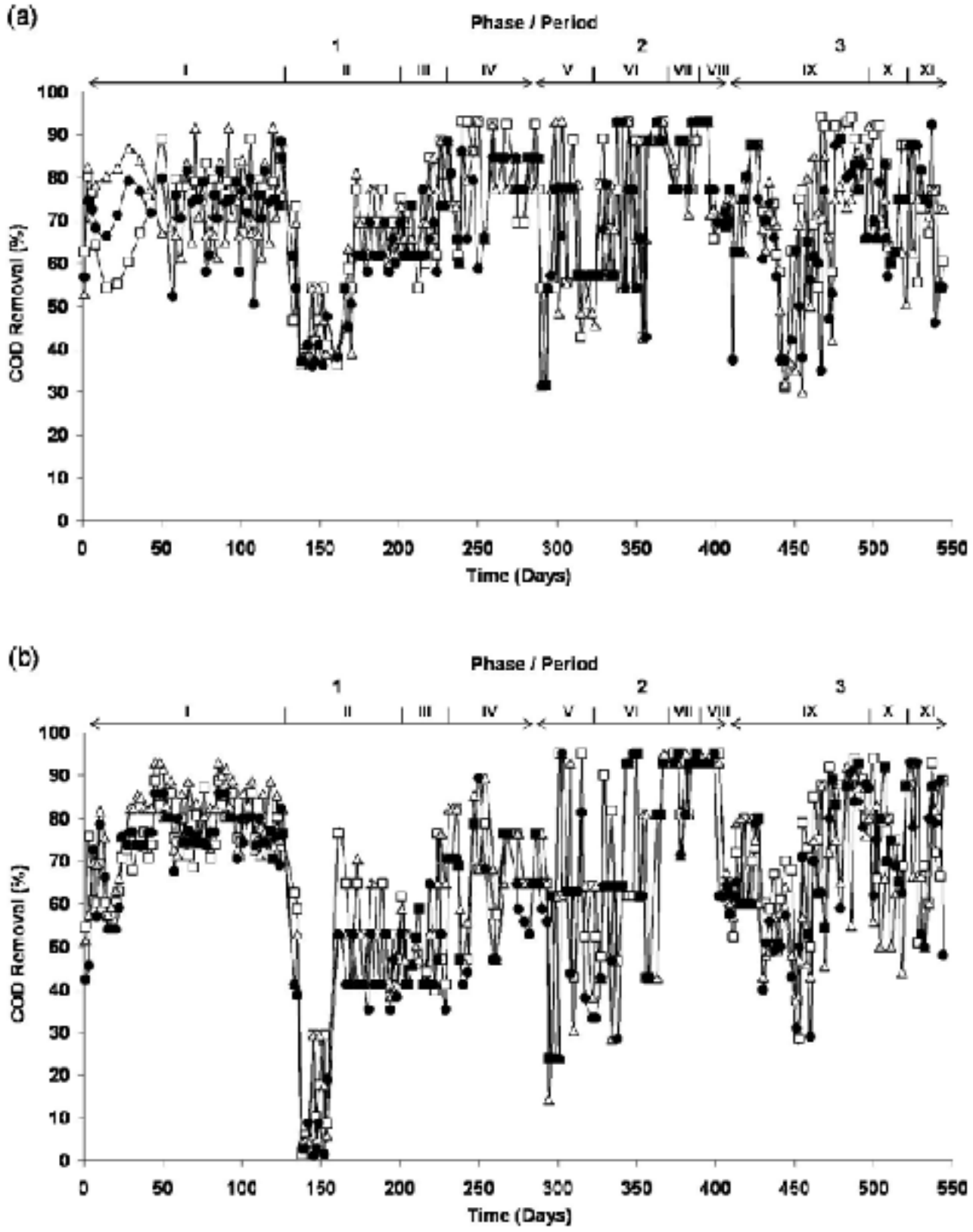


Figure 2.2. (a) Total and (b) soluble COD removal efficiencies for R1–R3 during the trial. R1 = 37˚C (❍); R2 = 15˚C (Δ); R3 = 10˚C (●).

2.2.2 Bioreactor Performance: Phase 2

The reduction in influent COD (mg l^{-1}) concentration of Phase 2 (Table 2.1) had an immediate effect on performance and by day 4 of PV, COD_{tot} had dropped to 54% (R1) and 31% (R2, R3) (Fig. 2.2a). Biogas quality values R1–R3 reflected this drop in removal efficiencies (Fig. 2.3a). The remainder of PV and PVI was characterised by erratic performance for R1–R3 (Figs 2.2a and b). However, the mean COD removal efficiencies for PVI were >72% (Fig. 2.2a) total and >67% (Fig. 2.1b) soluble. Biogas quality improved in tandem with the improved bioreactor performance with an average CH_4 content of biogas *c.* 50% (Fig. 2.3a). The erratic removal rates observed for all bioreactors for PV–PVI were replaced by much more stable operation during PVII (OLR, 2 kg COD m^{-3} d^{-1}), with mean COD total and soluble at 83–90% (Figs 2.2a and b). The final operational change (PVIII) of Phase 2 involved a reduction in HRT to 1.5 hr resulting in an OLR of 4 kg COD m^{-3} d^{-1}. The increased rate of influent resulted in a decrease in COD removal (Figs 2.2a and b). Nevertheless, mean COD removal efficiencies were >75% total and >81% (Figs 2.2a and b) soluble for all bioreactors. The biogas quality for PVII and VIII was comparable for all bioreactors, at 47–55% (Fig. 2.3a and b, Table 2.1). Phosphate (PO_4^{3-}) analysis of bioreactor effluent was carried out during PV–VII of Phase 2. Values obtained suggest the occurrence of anaerobic phosphate uptake/accumulation by the bioreactor biomass or an alternative mechanism for P attenuation in the bioreactor (Fig. 2.4b). Acetic acid concentrations remained low, averaging 0.09 mM ml^{-1} for all bioreactors (Fig. 2.4a).

2.2.3 Bioreactor Performance: Phase 3

The final phase of operation saw a return to the initial influent concentration of 750 mg total COD l^{-1}. PIX–XI recreated the operational parameters applied in PI–III of Phase 1, although over a shorter timeframe. The reintroduction of the higher-strength influent resulted in an immediate effect on removal efficiencies. A sharp decrease in performance from day 440 heralded a phase of erratic COD_{tot} removal efficiency R1–R3 (30–94%; Fig. 2.2a), with COD_{sol} removal efficiency displaying similar inconsistency (Fig. 2.2b). Performance recovered and stabilised over the latter half of PIX. The inconsistent performance which was a feature of PIX was replaced by more consistent removal during PX with COD_{tot} of >87% being achieved for all bioreactors by completion of the period (Fig. 2.2a). COD_{sol} displayed similar consistency, with R1–R3 mean values of 79%, 68% and 76% respectively (Fig. 2.2b). The final operational change of the current study involved a decrease of HRT to 3 h, resulting in an OLR of 6 kg COD m^{-3} d^{-1} (PXI). The increased loading rate had no significant adverse effect on performance (Figs 2.2a and b). Biogas quality improved during Phase 3 as a result of the increased influent strength, R1 68%, R2 61% and R3 59% mean biogas quality (Fig. 2.3a). Greater fluctuations were observed in acetic acid concentrations (0–0.31 mM ml^{-1}) than recorded for the previous phases. However, mean values for all bioreactors remained low (0.08 mM ml^{-1}, Fig. 2.4a).

2.2.4 SMA Profiles of Seed Inoculum

The specific methanogenic activity (SMA) values obtained for the seed biomass at the lower test temperatures contrasted greatly with those observed at 37°C, indicating the higher potential activity at 37°C (Table 2.2). All substrates showed a marked decrease in activity at 15°C and a further decrease at 10°C. Indirect substrates displayed significant decreases in potential activity to those obtained at 37°C with a 10- and 13-fold decrease in activity against ethanol, a 4- and 6-fold decrease against propionate and a 4- and 12-fold decrease against butyrate being recorded at 15°C and 10°C respectively (Table 2.2). Values against direct methanogenic substrates showed a similar trend with a 7-and 8-fold decrease against acetate and a 2- and 8-fold decrease against H_2/CO_2 at 15°C and 10°C respectively (Table 2.2, Fig. 2.6). Recorded values for the direct methanogenic precursors, acetate and H_2/CO_2 indicated the dominance of acetoclastic methanogens. This was the case for all test temperatures, with an almost 6:1 ratio in activity of acetoclastic:hydrogenothrophic methanogens being recorded at 37°C and 10°C (Table 2.2, Fig. 2.6a).

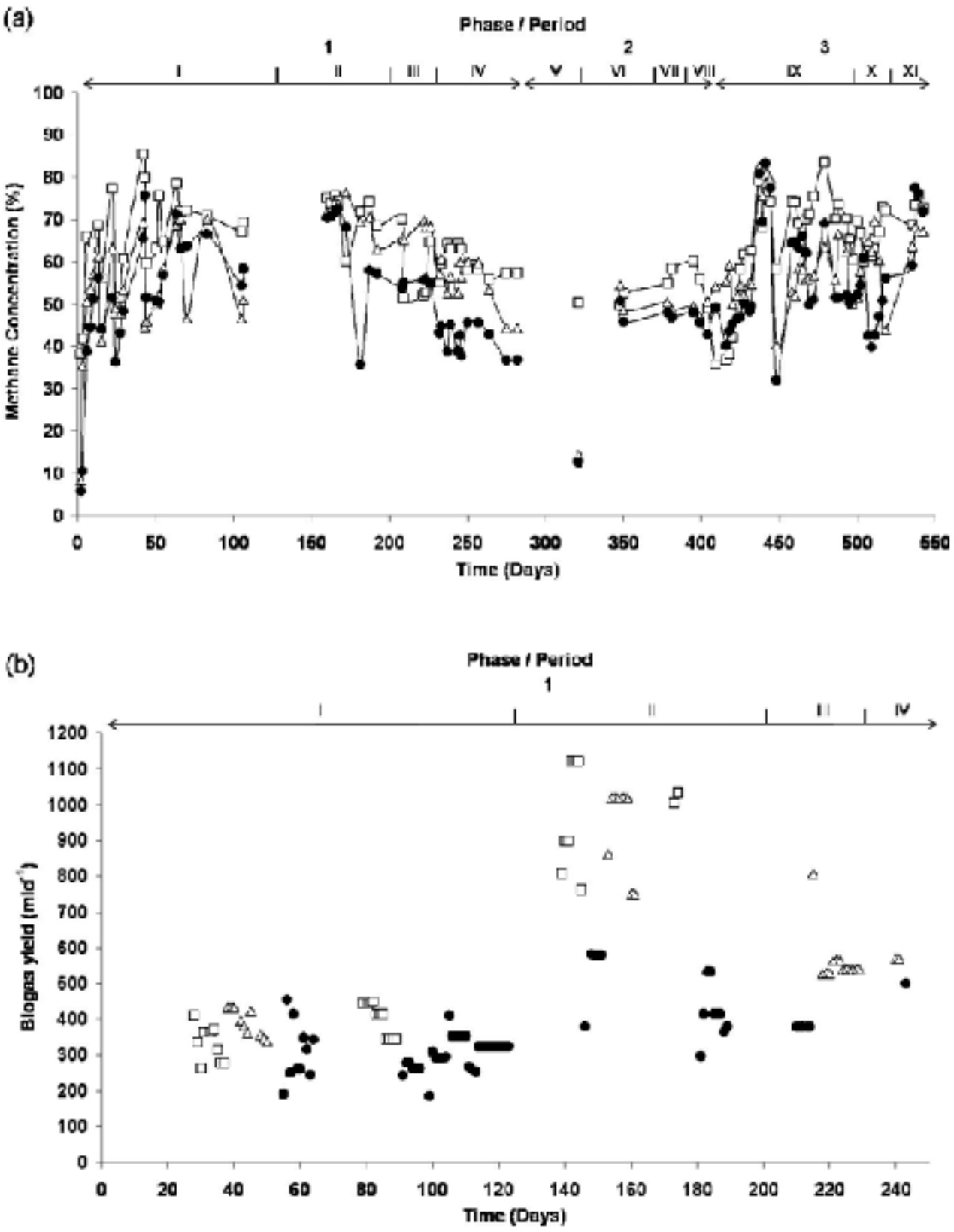


Figure 2.3. Biogas methane concentration (a) and biogas yield (b) for R1–R3 during trial. R1 = 37˚C (O); R2 = 15˚C (Δ); R3 = 10˚C (●).

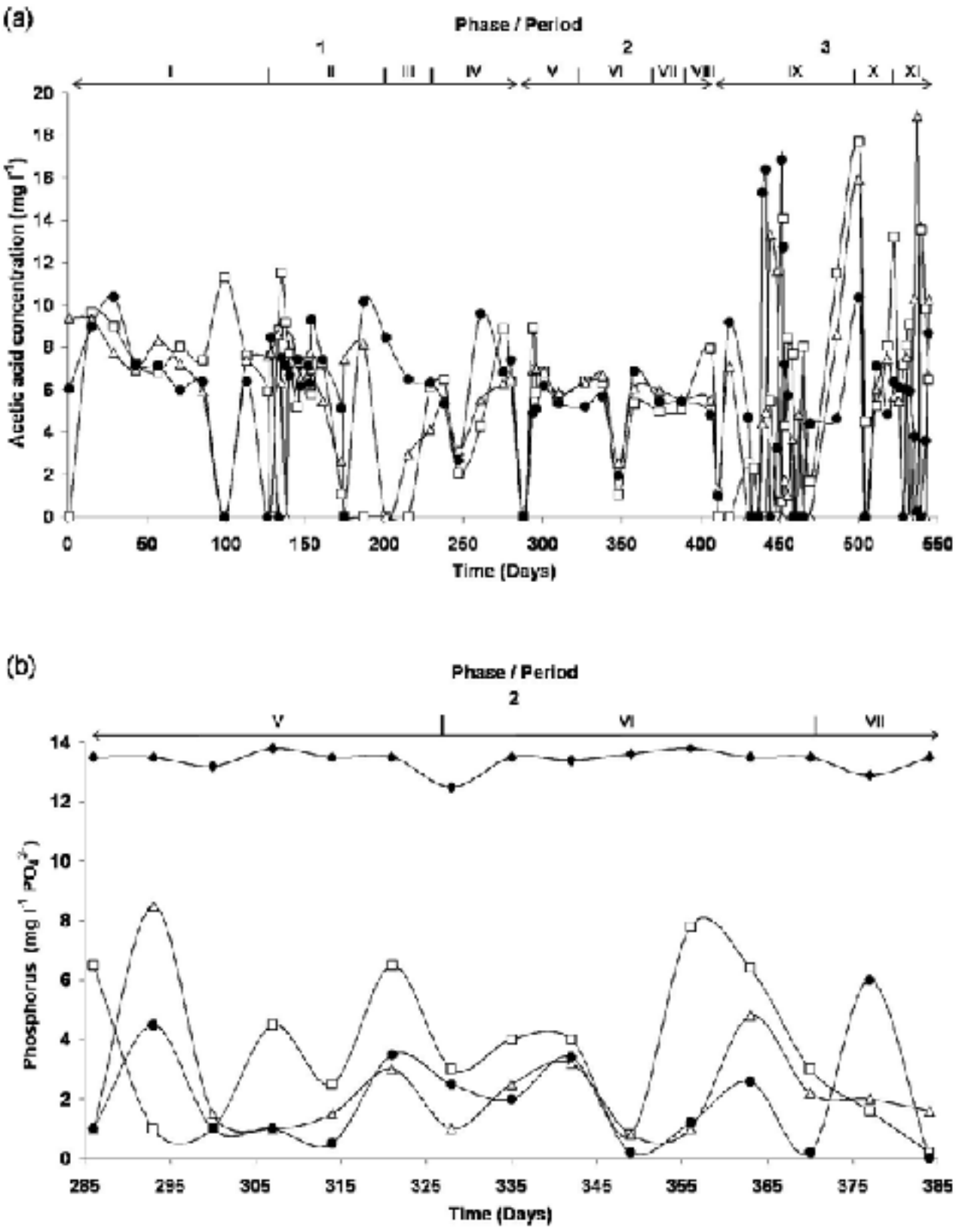


Figure 2.4. Acetate (a) and total phosphate (b) concentrations for effluents sampled from R1–R3 during trial. R1 (⭘); R2 (∆); R3 (●), influent phosphate (◆).

Table 2.1. Operational and performance characteristics of bioreactors R1, R2 and R3.

Phase		1				2				3		
Period		**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**
Days		0–124	125–201	202–230	231–285	286–325	326–371	372–392	393–405	406–500	501–525	526–544
Influent COD(T) [a]		750 ±50	750 ±50	750 ±50	750 ±50	250 ±40	250 ±40	250 ±40	250 ±40	750 ±50	750 ±50	750 ±50
Influent COD (S) [b]		240 ±30	240 ±30	240 ±30	240 ±30	80 ±20	80 ±20	80 ±20	80 ±20	240 ±30	240 ±30	240 ±30
HRT [c]		12	6	3	12	12	6	3	1.5	12	6	3
OLR [d]		1.5	3	6	1.5	0.5	1	2	4	1.5	3	6
VLR [e]		2	4	8	2	2	4	8	16	2	4	8
CODRE(T) [i]	R1	75 ±6	60 ±12	68 ±9	80 ±11	62 ±14	74 ±14	85 ±5	75 ±4	75 ±11	78 ±10	67 ±8
	R2	74 ±8	60 ±12	71 ±7	79 ±9	65 ±15	76 ±12	83 ±9	76 ±2	69 ±12	67 ±7	75 ±3
	R3	72 ±6	56 ±11	66 ±7	77 ±11	64 ±10	72 ±13	86 ±5	78 ±4	66 ±12	73 ±9	71 ±14
CODRE(S) [j]	R1	75 ±6	45 ±18	48 ±13	67 ±9	64 ±12	68 ±10	91 ±5	88 ±16	71 ±13	79 ±7	71 ±10
	R2	79 ±8	42 ±17	52 ±14	70 ±9	58 ±19	69 ±17	89 ±7	88 ±16	64 ±14	68 ±11	74 ±13
	R3	74 ±7	36 ±16	46 ±11	63 ±11	53 ±18	68 ±17	90 ±7	81 ±13	64 ±14	76 ±9	72 ±14
CH4 [k]	R1	65 ±9	72 ±4	58 ±7	60 ±3	50 ±0	51 ±1	57 ±2	55 ±4	64 ±10	66 ±4	73 ±2
	R2	52 ±9	71 ±3	67 ±2	55 ±4	14 ±0	52 ±3	50 ±1	50 ±1	58 ±7	59 ±6	67 ±1
	R3	50 ±11	63 ±10	55 ±1	42 ±3	13 ±0	48 ±3	48 ±1	46 ±2	56 ±10	49 ±6	71 ±6

All values are the period mean with ± period standard deviation; [a] Chemical oxygen demand, total (mg l^{-1}); [b] Chemical oxygen demand, soluble (mg l^{-1}); [c] Hydraulic retention time (h); [d] Organic loading rate (kg COD m^{-3} d^{-1}); [e] Volumetric loading rate (m^3 Wastewater m^{-3} Reactor d^{-1}); [f] Sludge loading rate (kg COD kg[VSS]$^{-1}$ d^{-1}); [g] Sludge loading rate ($m^3_{Wastewater}$ kg[VSS]$^{-1}$ d^{-1}); [h] Up-flow velocity (m h^{-1}); [i] Total COD removal efficiency (%); [j] Soluble COD removal efficiency (%); [k] Methane in biogas (%).

2.2.5 SMA Profiles of Bioreactor Biomass: Phase 1

A significant alteration in the structure of the methanogenic community of biomass samples from each bioreactor was observed on day 124 with a marked increase in hydrogen-utilising activity; these increases were coupled with decreases against acetate. This trend was most striking for the R1 (37°C) biomass (Fig. 2.5a). The structure of the R1 methanogenic community following the initial shift indicated from SMA testing on day 124 averaged 2:1 hydrogenotrophic:acetoclastic methanogenic activity. This hydrogenotrophic methanogen dominance of R1 was not a feature of the lower temperature R2 (15°C) and R3 (10°C) biomass (Figs 2.6a and b). By the conclusion of phase 1, SMA values obtained for the indirect substrates ethanol, propionate and butyrate of all bioreactors were lower than those recorded for the seed inoculum. These decreases were most distinct for assays against propionate with a 78, 86 and 92% decrease in potential activity for R1, R2 and R3 respectively from values obtained for the seed inoculum (Fig. 2.5a–c).

2.2.6 SMA Profiles of Bioreactor Biomass: Phase 2

Specific methanogenic activity testing on completion of the initial period (PV) of Phase 2 (day 325) displayed decreases by all bioreactors for both the acetate and H_2/CO_2 assays (Fig. 2.5a–c), with the exception of the R2 biomass against H_2/CO_2 (Fig. 2.5b). This downward trend ceased and values recovered to a degree from this point to the completion of the phase, with the R2 biomass again opposing the trend. After an initial increase in activity against H_2/CO_2, reduced values

Table 2.2. Specific methanogenic activity (ml CH_4 g [VSS]$^{-1}$ d^{-1}) of bioreactor biomass.

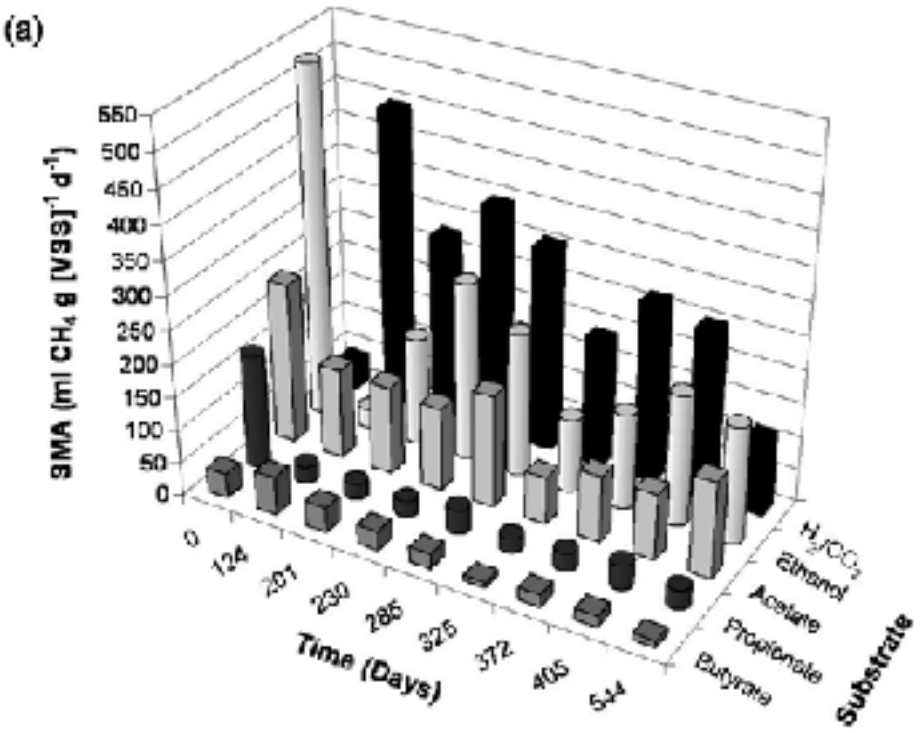
Day	Biomass	Test temp. (°C)	Ethanol	Acetate	Propionate	Butyrate	H_2/CO_2
0	Seed	37°C	528 ±5	243 ±15	171 ±8	37 ±3	42 ±2
	Inoculum	15°C	51 ±2	35 ±2	43 ±4	9 ±1	20 ±1
		10°C	38 ±4	29 ±1	28 ±2	3 ±1	5 ±1
405	R1 GSB	37°C	197 ±1	100 ±5	37 ±1	17 ±1	252 ±5
		15°C	18 ±3	24 ±2	10 ±3	16 ±4	27 ±2
		10°C	NAD	5 ±1	NAD	NAD	7 ±1
	R2 GSB	37°C	293 ±4	177 ±5	66 ±2	61 ±4	168 ±4
		15°C	35 ±8	27 ±2	22 ±4	22 ±5	24 ±2
		10°C	9 ±1	13 ±2	5 ±1	12 ±1	12 ±2
	R3 GSB	37°C	196 ±5	82 ±9	24 ±5	42 ±1	126 ±5
		15°C	24 ±1	34 ±5	26 ±2	35 ±3	25 ±1
		10°C	8 ±1	14 ±4	5 ±1	7 ±2	21 ±1
544	R1 GSB	37°C	176 ±13	145 ±5	29 ±2	10 ±2	114 ±14
		15°C	24 ±1	20 ±2	4 ±1	2 ±1	19 ±3
		10°C	14 ±3	10 ±1	3 ±1	2 ±1	9 ±1
	R1 AF	37°C	139 ±13	70 ±7	27 ±5	17 ±8	188 ±17
	R2 GSB	37°C	183 ±5	203 ±9	69 ±4	52 ±7	100 ±5
		15°C	50 ±6	56 ±5	8 ±1	9 ±3	29 ±2
		10°C	34 ±3	32 ±1	6 ±1	6 ±1	22 ±2
	R2 AF	15°C	41 ±2	45 ±3	8 ±1	11 ±1	29 ±1
	R3 GSB	37°C	227 ±21	198 ±8	85 ±2	50 ±5	130 ±6
		15°C	40 ±1	48 ±5	18 ±1	11 ±1	31 ±5
		10°C	28 ±7	30 ±2	6 ±1	6 ±1	13 ±1
	R3 AF	10°C	25 ±1	26 ±1	6 ±1	7 ±1	13 ±1

All values are the mean of triplicates ± standard deviation; GSB – granular sludge bed biomass; AF – anaerobic filter biomass; NAD – no activity detected.

were recorded for R2 on days 372 and 405 (Fig. 2.5b). On completion of Phase 2, decreased values were recorded against both direct methanogenic substrates (acetate and H_2/CO_2) for all bioreactors from values obtained on completion of Phase 1. Fluctuations were observed against indirect substrates: however, on completion of Phase 2 most potential SMA values had recovered to pre-phase rates (Fig. 2.5a–c). R2 and R3 on day 405 returned higher potential activity values against propionate and butyrate than those recorded at the commencement of Phase 2 (Figs 2.5b and c). R2 and R3 potential activity against butyrate and H_2/CO_2 displayed higher activity values than those obtained from the seed inoculum, indicating the development of a low-temperature tolerant mesophilic biomass (Fig. 2.5, Table 2.2). On day 405, the completion of Phase 2 SMA testing of biomass from each bioreactor was carried out at 37, 15 and 10°C. All samples displayed highest potential activity at 37°C (Fig. 2.6, Table 2.2), indicating that the biomass had retained a mesophilic, though psychrotolerant, nature despite 405 days of operation at 15 and 10°C in the case of the R2 and R3 biomass respectively. R1 returned no activity against the indirect methanogenic substrates ethanol, propionate and butyrate at 10°C (Table 2.2).

2.2.7 SMA Profiles of Bioreactor Biomass: Phase 3

Specific methanogenic activity testing of bioreactor biomass samples at their respective operational temperature on completion of Phase 3 (day 544) displayed a significant increase in acetoclastic methanogenic activity for all samples, with R2 and R3 displaying a 2-fold increase in potential activity against acetate. Decreases in activity against H_2/CO_2 were exhibited for the R1 and R3 biomass while R2 showed a slight increase against H_2/CO_2. Values against indirect substrates generally displayed a decrease in activity from values obtained on day 405, with the exception of R2 and R3 activity against ethanol, R3 showed a >3-fold increase (Fig. 2.5). Once again, when bioreactor biomass samples from R2 and R3 were tested at increased temperature, activity increased dramatically (Fig. 2.6b and c; Table 2.2). Specific methanogenic activity testing was carried out on the fixed film biomass recovered from the AF at bioreactor operational temperature. Anaerobic filter SMA against all substrates was similar to the granular sludge bed biomass for the lower-temperature R2 and R3. R1 displayed reduced activity against indirect substrates with the exception of butyrate; a 50% reduced activity against acetate was also observed. However, greater activity against H_2/CO_2 was recorded within R1 AF biofilm compared with the sludge bed biomass (Table 2.2).



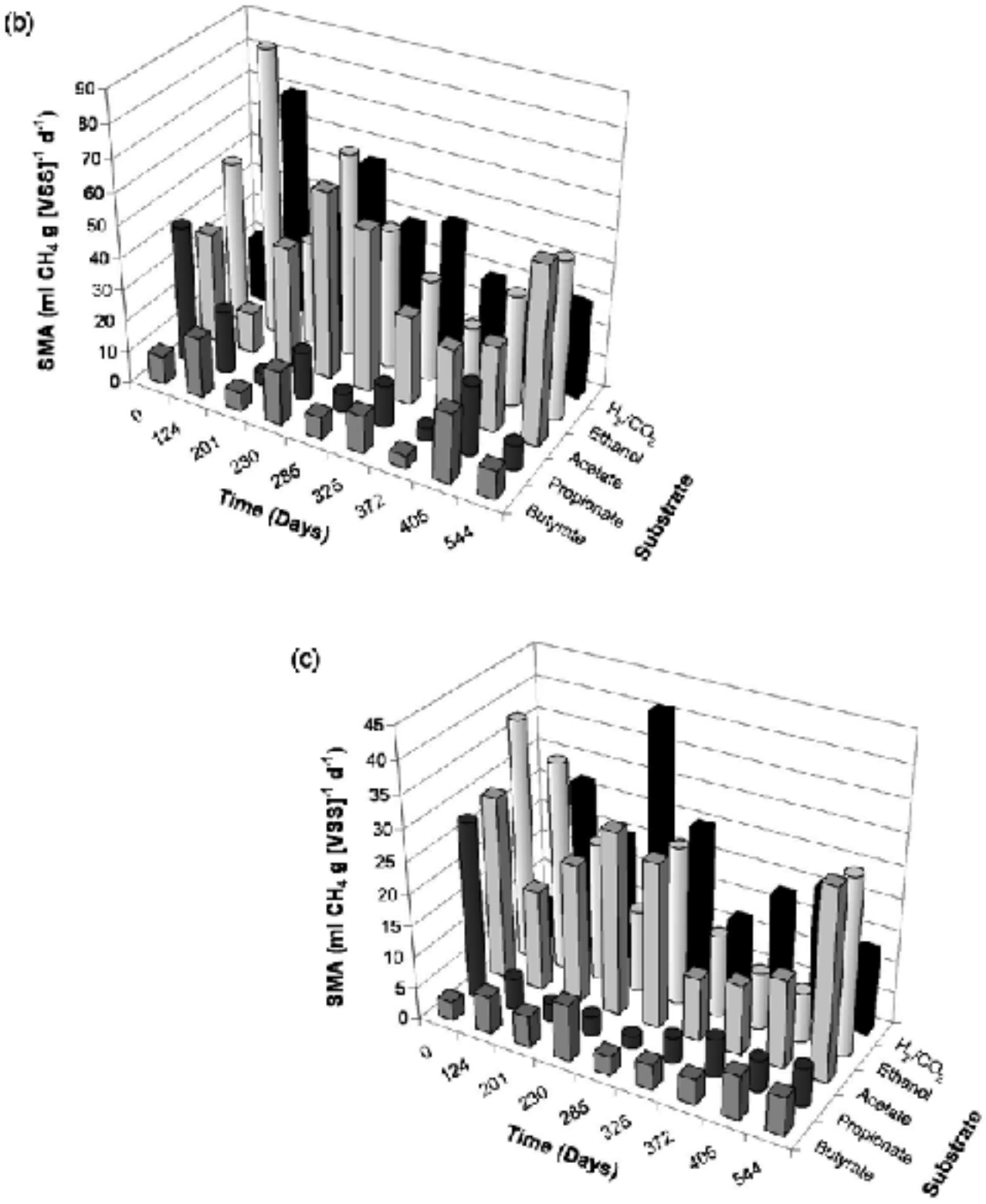
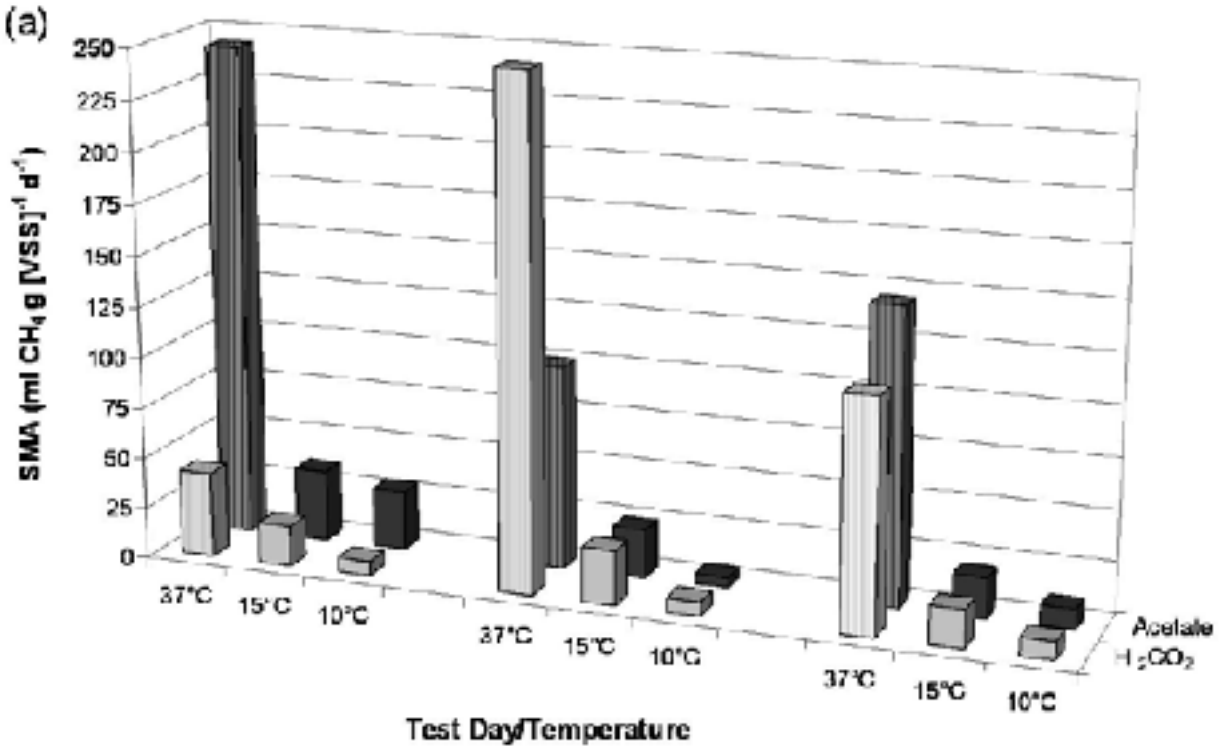
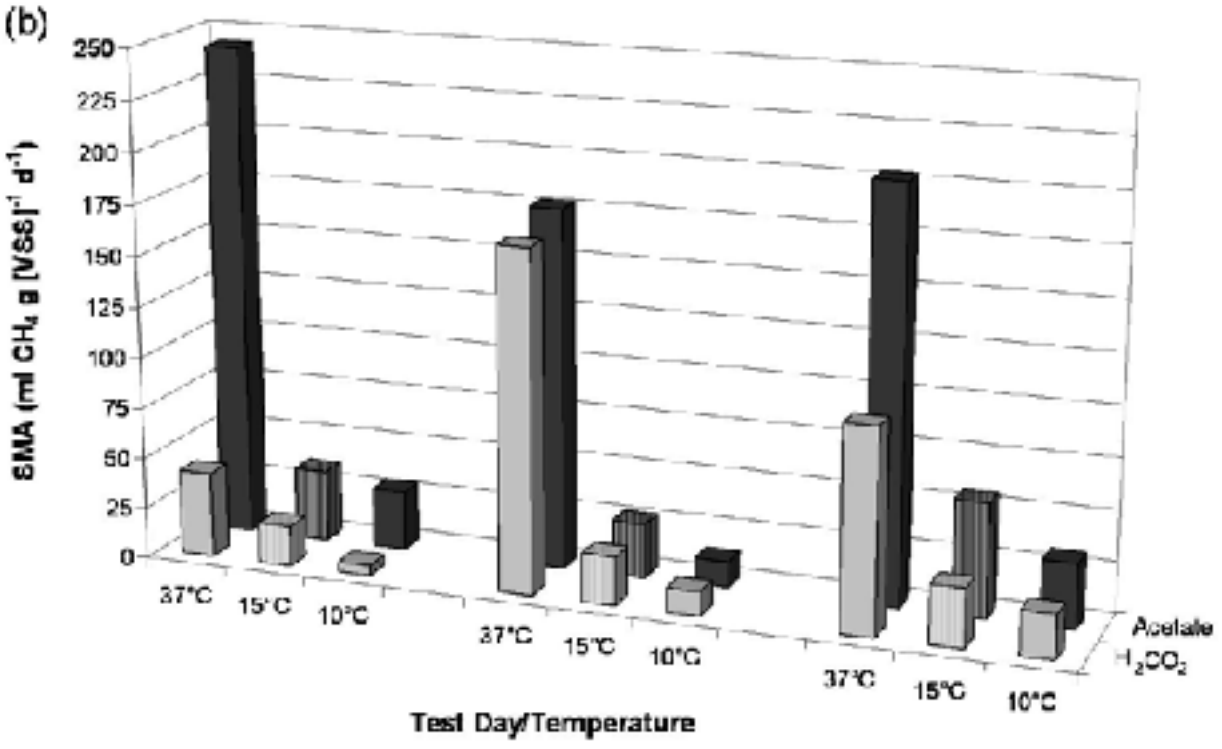


Figure 2.5. Specific methanogenic activity (SMA) profiles for sludge bed biomass sampled from R1 (a; 37˚C), R2 (b; 15˚C) and R3 (c; 10˚C) during trial.


(a)
SMA (ml CH_4 g [VSS]$^{-1}$ d^{-1})
250
225
200
175
150
125
100
75
50
25
0
37°C
15°C
10°C
37°C
15°C
10°C
37°C
15°C
10°C
Acetate
H_2CO_2
Test Day/Temperature


(b)
SMA (ml CH_4 g [VSS]$^{-1}$ d^{-1})
250
225
200
175
150
125
100
75
50
25
0
37°C
15°C
10°C
37°C
15°C
10°C
37°C
15°C
10°C
Acetate
H_2CO_2
Test Day/Temperature

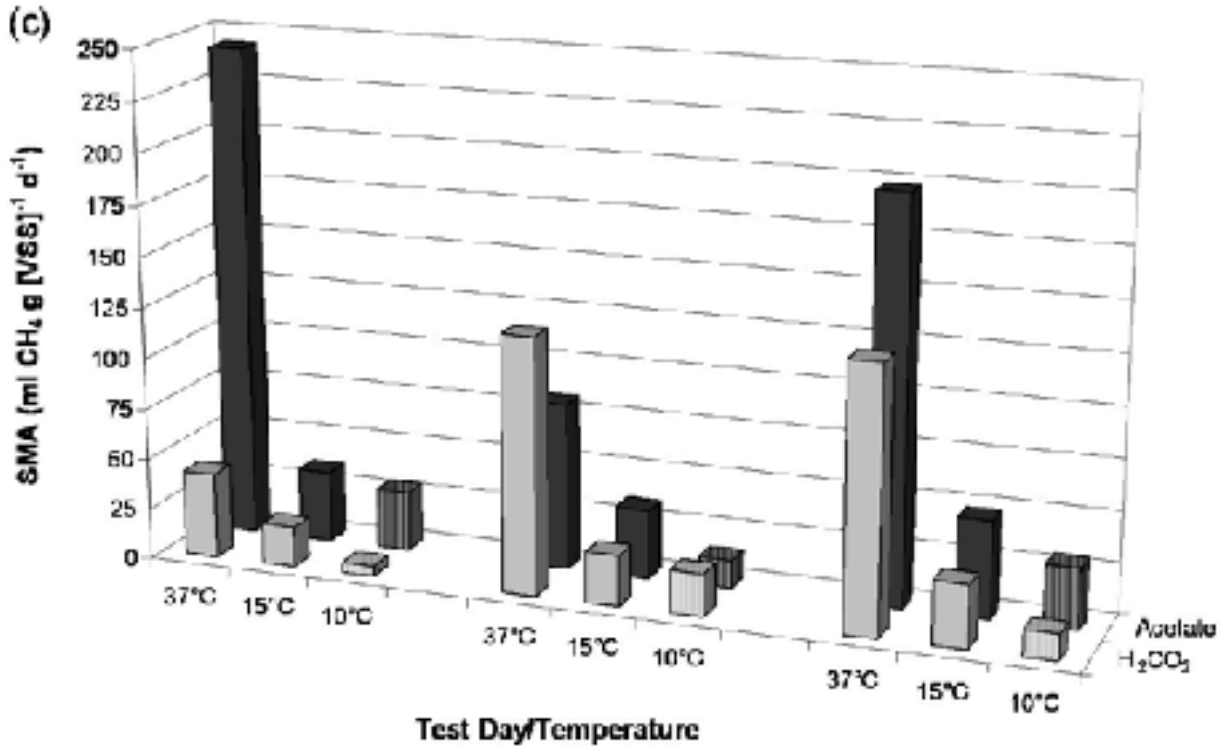


Figure 2.6. Effect of temperature on specific methanogenic activity (SMA) profiles of sludge bed biomass sampled from R1 (a), R2 (b) and R3 (c) during trial.

2.2.8 Microbial Community Structure and Dynamics in R1–R3 Biomass

Archaeal 16S rRNA gene clone library analysis of all samples throughout the reactor trials revealed that the methanogens included a high abundance of *Methanosaeta*-like clones (*c.* 65–75%, Fig. 2.7a), followed by members of the *Methanobacteriales* group. Representatives of the Group 1.3 *Crenarchaeota* were found to be present in the seed sludge and also prevailed throughout the trials, suggesting a putative role of these non-methanogens in low-temperature sewage treatment. The presence of these organisms in anaerobic digesters has been documented previously, and a homoacetogenic role in concert with acetoclastic methanogens hypothesised (Collins et al., 2005). It is possible that crenarchaeal growth in this trial was limited by competition from hydrogenotrophic methanogens.

Bacterial communities, as deduced from clone library analysis, were diverse and dynamic, especially by the end of the trial, when the presence of Planctomycete-like, TM7 and OP11-like clones was recorded (Fig. 2.7b). Overall, libraries were dominated by the Bacteroides, Firmicutes, Spirochaete and Chloroflexi groups with the emergence of different proteobacterial members by the end of the trial. The diversity of bacterial phyla present in the sewage-degrading biomass is most likely a reflection of the complex nature of the sewage-based wastewater and the existence of a greater level of functional redundancy amongst the bacteria – i.e. many different organisms can degrade, for example, amino acids, and one or more of these numerous groups can be favoured by slight changes in environmental conditions.

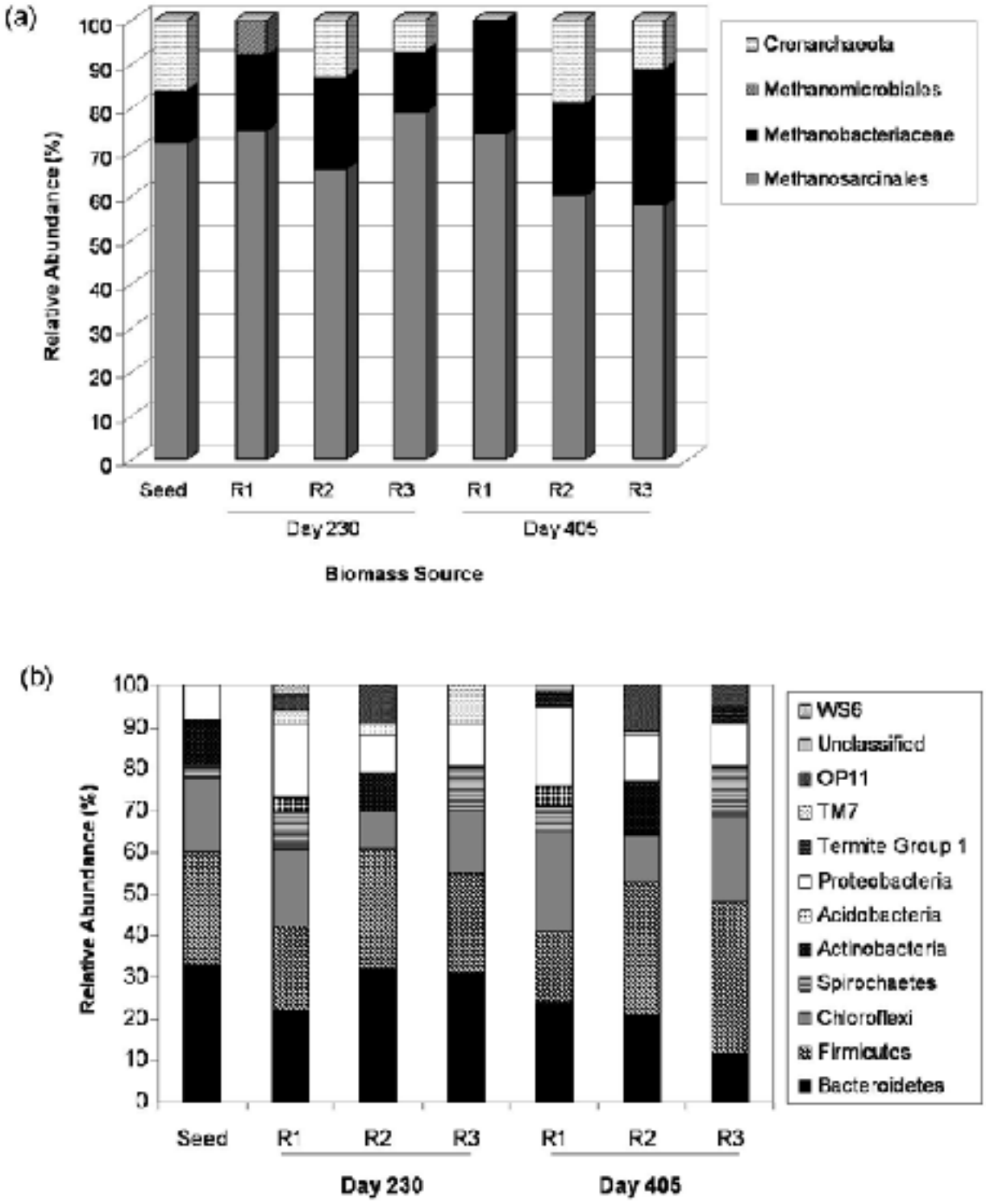


Figure 2.7. Relative abundance (%) of archaeal (a) and bacterial (b) clones analysed from seed biomass and from biomass samples collected from each bioreactor (R1–R3) on days 285 (TA) and 405 (TB).

2.3 Conclusions

- Low-temperature anaerobic digestion of synthetic sewage-based wastewater was successfully applied at OLRs of up to 6 kg COD m^{-3}, day^{-1} and HRT as low as 1.5 h, with average and stable total COD removal efficiencies of 70% under these conditions.
- The SMA profiles for the AF sections of the low-temperature reactors indicated that the biomass was as active and important, with respect to COD removal, as that in the granular sludge section. This was not the case, to as great an extent, in the mesophilic reactor.
- The high level of bacterial biodiversity, with the associated robustness in response to changing environmental conditions, along with the stable maintenance of methanogenic populations in the system, suggests that the long-term operation of a low-temperature anaerobic bioreactor is feasible from a microbiological point of view, a suggestion strongly supported by the SMA profiles taken during the reactor trials.
- High levels of phosphate attenuation were noted during the trials (up to 80% P removal) – this is a significant feature of the process, but the mechanism involved remains unclear.
- The results presented in this section suggested that a modified reactor configuration, with enhanced features, allowing greater biomass retention and biofilm formation, could benefit the performance and efficiency of low-temperature AD of sewage. The results of a trial to test this hypothesis are presented in Section 3.

3 Low Temperature (12°C) Anaerobic Treatment of Synthetic Sewage in Novel Anaerobic Hybrid Bioreactors

The results presented in Section 2 indicated that the fixed-biofilm section of the anaerobic hybrid reactor could play an important role in COD removal during LTAD of sewage. This was in contrast to the situation reported previously for LTAD of high-strength industrial wastewaters (Collins et al., 2005a, b, c; McKeown et al., 2009), where >90% of the COD removal occurred in the granular sludge bed section. This section presents the results of a trial that investigated using an alternative bioreactor design, with improved filtration capacity, to enhance the efficiency and stability of LTAD of sewage. The principal hypothesis being tested was that long-term, low-temperature (12°C) anaerobic treatment of synthetic sewage could be achieved successfully, with improved operational performance and stability, to that achieved using the conventional anaerobic hybrid reactors employed for the trials described in Section 2. A chemically defined, synthetic sewage (SYNTHES; Aiyuk and Verstraete, 2004) was employed for these experiments.

3.1 Experimental

3.1.1 Source of Biomass

Anaerobic sludge granules were obtained from a full-scale, mesophilic (37°C), IC bioreactor at Carberry Milk Products (Ballineen, Co. Cork, Ireland) used to treat dairy-production process wastewaters. The VSS concentration of the granules was 102.8 g VSS l^{-1}.

3.1.2 Bioreactor Design and Operation

Two glass, laboratory-scale (3.5 l; active liquid volume, 3.0 l) bioreactors, R4 and R5, were used in the present study. R4 and R5 were of a novel design and were operated as duplicates. Each bioreactor was inoculated with 20 g VSS $l_{reactor}^{-1}$ and was operated at 12°C (the average temperature of sewage in Ireland). R4 and R5 were employed to treat a synthetic sewage wastewater (SYNTHES; Aiyuk and Verstraete, 2004) at a concentration of 500 mg COD $l_{influent}^{-1}$. The trial was divided into 6 operating periods (PI–VI), which were characterised by changes in HRT and consequent OLR (Table 3.1). Effluent was recirculated through the respective bioreactors to give an applied liquid upflow velocity of 2.5 m h^{-1}.

3.1.3 Methanogenic Activity and Analytical Techniques

The SMA profiles were determined at 12˚C and 37°C, as described in Section 2, for the seed biomass (day 0), and of biomass from R4–R5 on days 120 and 282. Samples of R4 and R5 effluent were taken thrice weekly and analysed for total COD (COD_{tot}) and soluble COD (COD_{sol}), pH, biogas production rates and concentrations, as described in Section 2. Acetic acid concentrations were determined by GC-MS (Varian, USA). Phosphate (PO_4^{3-}) was detected using a colorimetric spectrophotometer (HACH DR4000), according to the HACH molybdovanadate method with acid persulfate digestion.

3.1.4 Methanogenic Community Structure of R4 and R5 Biomass

The methanogenic community structure was determined for R4 and R5 biomass samples at the conclusion of the trial (day 282) by 16S rRNA clone library analysis as described in Section 2.

3.2 Results and Discussion

3.2.1 Bioreactor Performance

A very short start-up period was observed for each of the bioreactors at the applied OLR of 0.5 kg COD m^{-3} d^{-1} (PI), with COD_{tot} and COD_{sol} removal efficiencies of *c.* 80% achieved by day 5 (Fig. 3.1a and b). This start-up regime compared favourably to that achieved during the first trials (Section 2) and also to literature guidelines for conventional mesophilic AD (McHugh et al., 2003). Stable COD removal efficiency of >80% continued throughout PI, for both R4 and R5, with effluent COD concentrations of <100 mg l^{-1}, and acetic acid could not be detected in effluent samples. Biogas methane concentrations increased during the first 25 days of operation and reached equilibrium by the end of PI, as the reactor headspace gas was displaced by biogas produced by the R4 and R5 biomass (Fig. 3.2a).

As summarised in Table 3.1, PII–VI involved an increase in the organic and volumetric loading rates applied to R4 and R5 through a stepwise reduction in HRT from 24 h down to 2 h. Through this period, the average total and soluble COD removal efficiencies of R4 and R5 were >70% and >65%, respectively (Table 3.1). The addition of the novel fixed-film packing material to the AF sections of the bioreactors greatly enhanced the stability of the process (Fig. 3.1), and much less fluctuation in day-to-day performance was encountered than during the first trials (Section 2; Fig. 2.2). This was reflected in the much narrower standard deviation range for both total and soluble COD removal efficiency during each of the six operational periods (10–15%; Table 3.1).

By the conclusion of the trial, both R4 and R5 had a total COD removal efficiency of 75%, with effluent values close to, or below, the discharge standard for total COD at an imposed OLR of 6 kg m^{-3} day^{-1} and a HRT of 2 h (Fig. 3.1; Table 3.1). Effluent acetic acid and other volatile fatty acids remained below the detection limit throughout the trial, while the quality of biogas improved to approximately 60% for both bioreactors by the end of the trial, as a result of the increased influent strength (Fig. 3.2a).

Phosphate (PO_4^{3-}) analysis of R4 and R5 bioreactor effluents was carried out throughout the trial (Fig. 3.2b). Values obtained confirmed the occurrence of anaerobic phosphate uptake/accumulation by the bioreactor

Table 3.1. Operational and performance characteristics during Periods I–VI.

Period		I	II	III	IV	V	VI
Days		**0-34**	**35-120**	**121-162**	**163-226**	**227-267**	**268-282**
Influent COD(T) [a]		497 ±18	504 ±25	526 ±52	512 ±30	496 ±18	516 ±22
Influent COD (S) [b]		293 ±12	280 ±22	302 ±26	282 ±31	274 ±34	286 ±24
HRT [c]		24	12	8	6	4	2
OLR [d]		0.5	1	1.5	2	3	6
VLR [e]		1	2	3	4	6	12
OSLR [f]		0.03	0.05	0.08	0.10	0.15	0.30
VSLR [g]		0.05	0.11	0.16	0.21	0.32	0.63
UV [h]		2.5	2.5	2.5	2.5	2.5	2.5
Effluent COD(T) [i]	R4	94 ±61	98 ±33	102 ±39	131 ±50	130 ±52	133 ±36
	R5	93 ±62	116 ±36	115 ±46	141 ±49	141 ±46	119 ±38
Effluent COD(S) [j]	R4	77±42	71 ±24	72 ±22	74 ±28	91 ±27	96 ±27
	R5	83 ±41	72 ±17	79 ±25	82 ±30	93 ±27	79 ±25
CODRE(T) [k]	R4	81 ±12	81 ±7	80 ±9	74 ±10	74 ±11	74 ±7
	R5	81 ±12	75 ±9	78 ±10	73 ±9	72 ±9	77 ±8
CODRE(S) [l]	R4	74 ±14	77 ±7	76 ±8	74 ±10	66 ±11	66 ±12
	R5	72 ±13	74 ±7	73 ±9	70 ±12	65 ±12	72 ±10
Influent phosphate (mgl^{-1} PO_4^{3-})		22 ±5	23 ±6	19 ±8	20 ±3	21 ±1	17 ±2
Effluent phosphate (mg l^{-1} PO_4^{3-})	R4	7 ±3	9 ±3	10 ±5	9.8 ±4.1	8 ±4	5 ±1
	R5	7 ±3	8 ±3	8 ±1	10 ±4	9 ±3	7 ±5
Methane in biogas (%)	R4	10 ±7	22 ±7	34 ±11	33 ±11	52 ±19	57 ±13
	R5	9 ±8	28 ±5	23 ±11	27 ±8	58 ±12	58 ±9

All values are the period mean with ± period standard deviation; a Influent chemical oxygen demand, total (mg l^{-1}); b Effluent chemical oxygen demand, soluble (mg l^{-1}); c Hydraulic retention time (hrs); d Organic loading rate (kg $CODm^{-3}$ d^{-1}); e Volumetric loading rate (m^3 Wastewater m^{-3} Reactor d^{-1}); f Sludge loading rate (kg COD kg[VSS]$^{-1}$ d^{-1}); g Sludge loading rate ($m^3_{Wastewater}$ kg[VSS]$^{-1}$ d^{-1}); h Up-flow velocity (m h^{-1}); i Total chemical oxygen demand (mg l^{-1}); j Soluble chemical oxygen demand (mg l^{-1}); k Total COD removal efficiency (%); l Soluble COD removal efficiency (%).

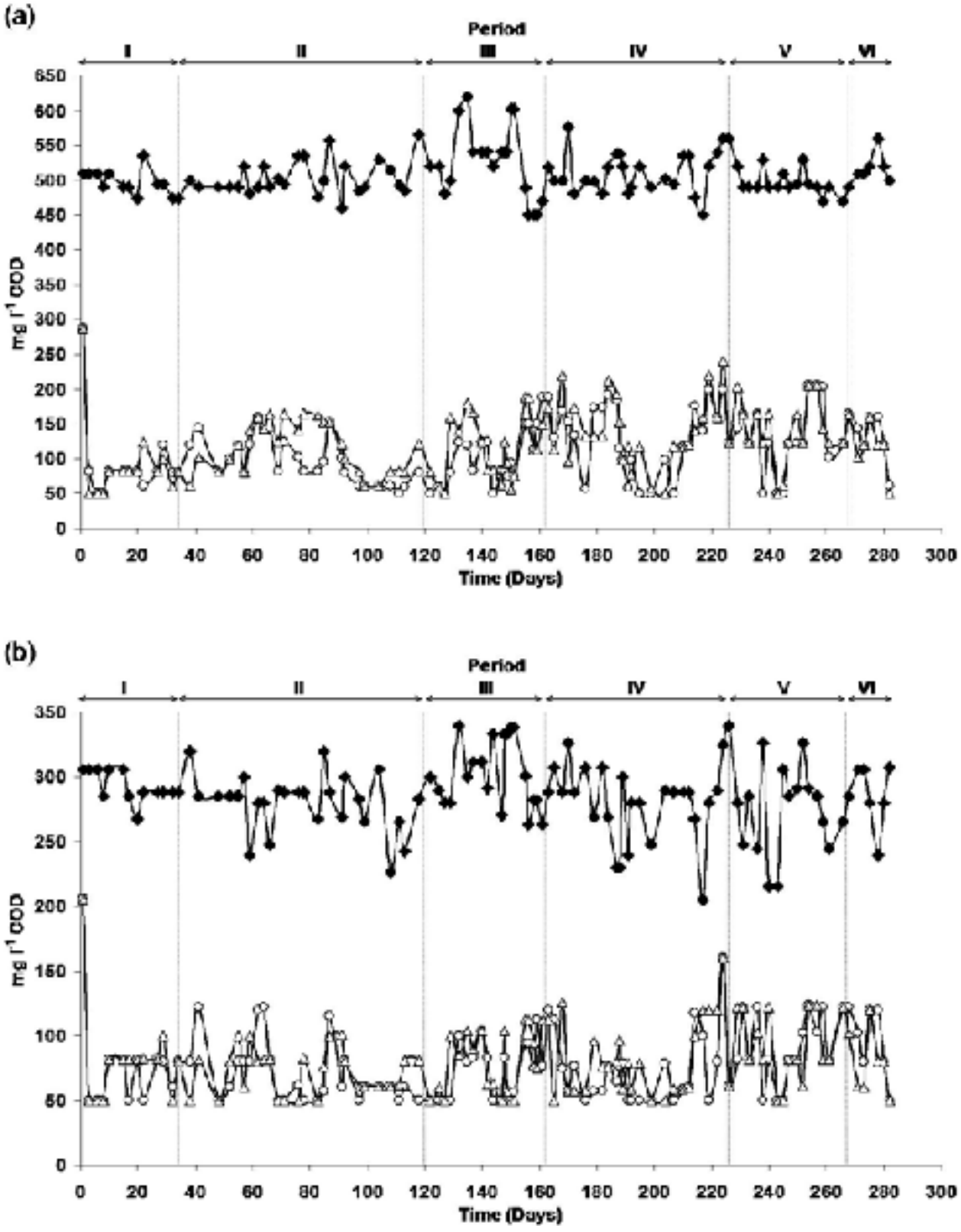


Figure 3.1. Total (a) and soluble (b) COD removal efficiency. Influent (◆). R 4(O), R5 (Δ).

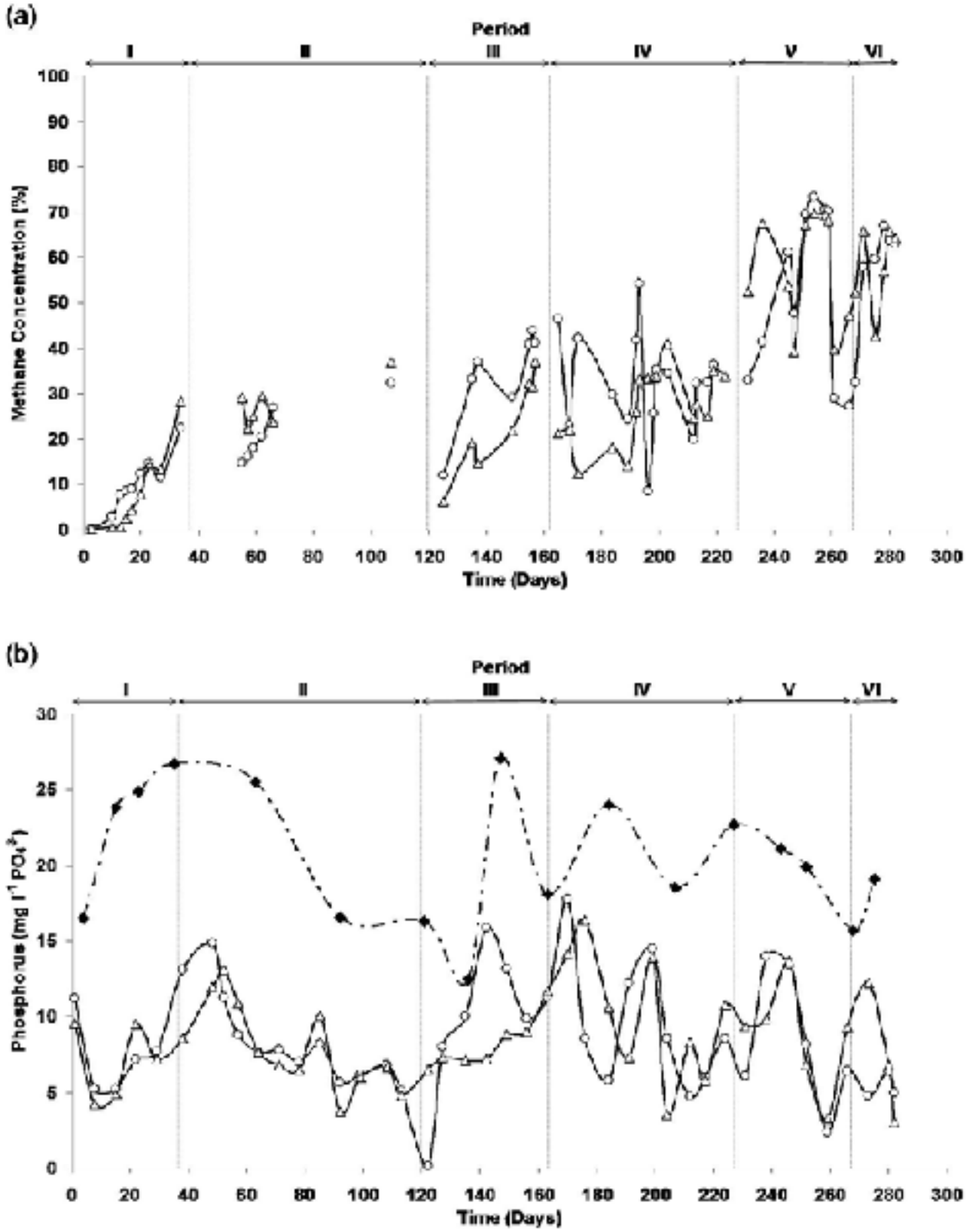


Figure 3.2. Biogas methane (a) and total phosphate (b) concentrations for effluents sampled from R4 and R5 during the trial. R4 (O); R5 (Δ); influent phosphate (◆).

biomass, or an alternative mechanism for P attenuation in the bioreactor first suggested by a partial analysis of effluents from the R1–R3 bioreactor trial (Fig. 2.4b). Mean P removal reached 60–70% during PII–V (Table 3.1), with lower values being recorded during start-up (PI) and during PVI when OLR was highest. This suggests a biological, rather than a purely physical, basis for phosphate attenuation in the system. This finding is clearly significant and represents a further potential advantage of the LTAD approach for sewage treatment.

3.2.2 SMA Profiles of Bioreactor Biomass

As reported in Section 2, SMA values obtained for the seed biomass at the lower test temperature contrasted greatly with those observed at 37°C, indicating the higher potential activity at 37°C (Table 3.2) and all substrates showed a marked decrease in activity at 12°C. In general, the SMA values, even at 37˚C, were lower than those recorded in Section 2, reflecting the fact that the seed inoculum had been stored for >6 months at 4˚C, between the two trials, and had clearly become less active during this period. Interestingly, however, the start-up of the bioreactors was not impacted by this reduced SMA – indicating that the reactivation of the biomass was rapid (Fig. 3.1a and b). Recorded values for the direct methanogenic precursors, acetate and H_2/CO_2 indicated the dominance of acetoclastic methanogens in the seed biomass (Table 3.2).

Specific methanogenic activity testing of bioreactor biomass samples at 12˚C and 37˚C on day 120 and at the conclusion of the trial on day 282 demonstrated the satisfactory development of methanogenic activity in both bioreactors with, for example, R4 displaying a 5-fold increase in potential activity against acetate at both temperatures tested by day 120 of the trial (Table 3.2). The SMA data from the R4 and R5 trials were in agreement with those reported in Section 2 for R1–R3, as all samples displayed highest potential activity at 37°C (Table 3.2), indicating that the biomass had retained a mesophilic, though psychrotolerant, nature. In general, the SMA profiles against the indirect substrates propionate and butyrate remained relatively low throughout the trial, again consistent with the findings from the first R1–R3 trials, reflecting perhaps the low concentrations of these compounds in the influent wastewater and the importance of acetate as a fermentation intermediate during anaerobic treatment of sewage (Tables 2.2 and 3.2).

Table 3.2. Specific methanogenic activity (ml CH_4 g [VSS]$^{-1}$ d^{-1}) of bioreactor(s) biomass.

Day	Biomass	Test temp. (°C)	Ethanol	Acetate	Propionate	Butyrate	H_2/CO_2
0	Seed	12°C	1.9 ±0.1	2.1 ±0.2	1.3 ±0.2	2 ±0.3	4.5 ±0.1
	Inoculum	37°C	7.1 ±1.1	19.4 ±2.2	1.8 ±0.6	3.2 ±1	11.1 ±0.5
120	R4 GSB	12°C	6.5 ±0.6	10.2 ±1.3	1.2 ±0.1	2.3 ±0.1	10.9 ±0.6
		37°C	28.6 ±3.4	96.2 ±5.2	7.3 ±2.1	14.1 ±3.2	48.6 ±7.2
120	R5 GSB	12°C	9 ±0.7	4.7 ±0.5	1.9 ±0.3	3.3 ±1.4	20.5 ±2.8
		37°C	43.6 ±12.1	51.6 ±3.8	8.3 ±2.7	19.8 ±4.3	109.7 ±6.9
282	R4 GSB	12°C	24.7 ±2.9	26.3 ±0.5	2.7 ±0.3	3.9 ±0.6	17.6 ±1.2
		37°C	181.2 ±3.2	247.2 ±6.8	16.1 ±3.7	31.6 ±3	163.4 ±25.6
282	R5 GSB	12°C	20.1 ±1.4	28.3 ±0.3	3 ±0.3	3.7 ±0.5	15.4 ±1.6
		37°C	143.9 ±4.1	278.8 ±17.5	14 ±1.7	22.9 ±1.7	118.7 ±27.6

GSB – granular sludge bed biomass; All values are the mean of triplicates ± standard deviation.

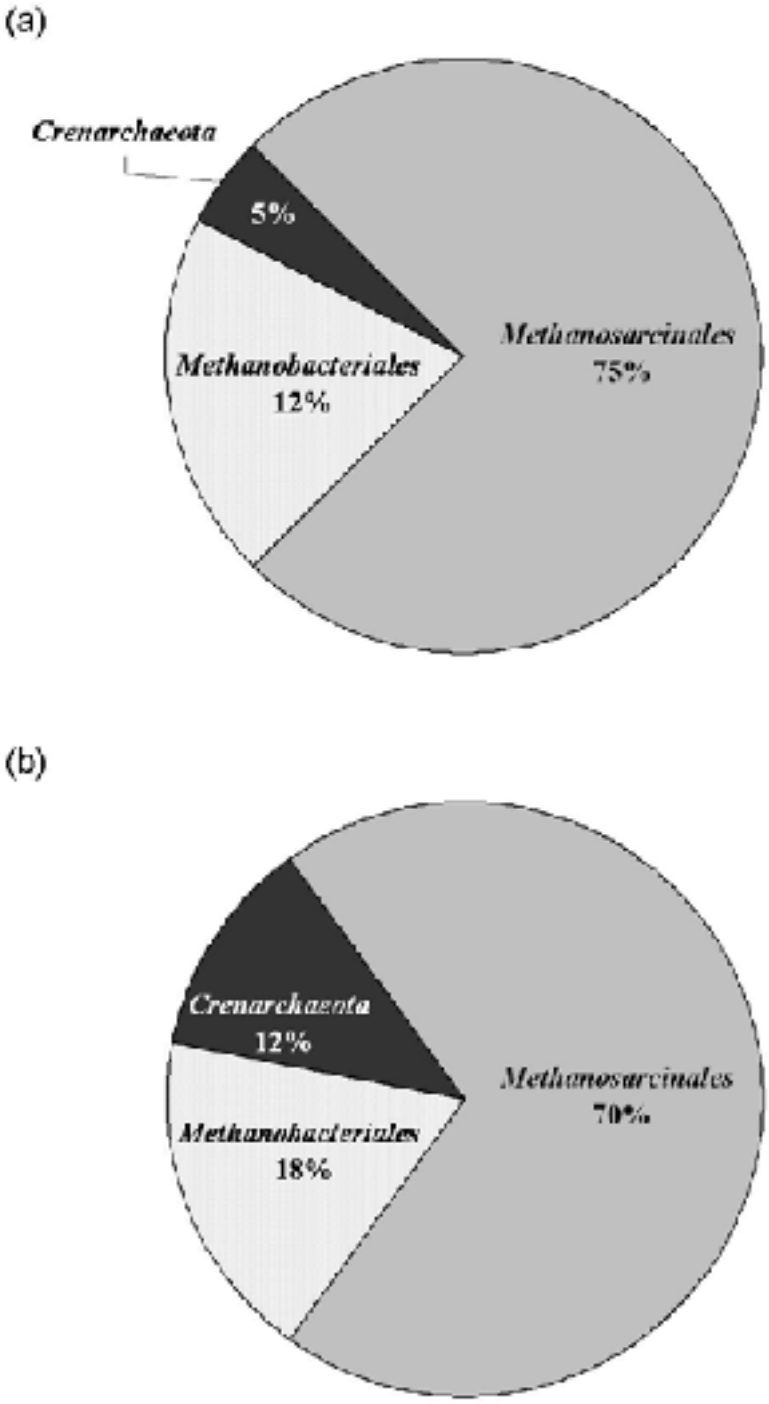


Figure 3.3. Relative abundance of Archaeal groups in 16S rRNA clone libraries from the R4 (a) and R5 (b) bioreactors sampled at conclusion of trial.

3.2.3 Microbial Community Structure

Archaeal 16S rRNA gene clone library analysis of all samples throughout the reactor trials revealed that the methanogens included a high abundance of acetoclastic *Methanosaeta*-like clones (*c.* 65–75%, Fig. 3.3a and b), followed by members of the hydrogenotrophic *Methanobacteriales* group. This is a similar finding to that observed in the R1–R3 trials described in Section 2.

3.3 Conclusions

- Low-temperature anaerobic digestion of synthetic sewage-based wastewater was successfully applied at OLRs of up to 6 kg COD m^{-3} day^{-1} and HRTs as low as 2 h. The addition of the modified AF section described in the section resulted in enhanced total COD removal efficiencies averaging >70% and very stable performance across the range of loading rates applied. Effluent total COD values were at, or below, the discharge standard throughout the trial.
- The high level of bacterial biodiversity, with the associated robustness in response to changing environmental conditions, along with the stable maintenance of methanogenic populations in the system, suggests that the long-term operation of a low-temperature anaerobic bioreactor is feasible from a microbiological point of view, a suggestion supported strongly by the SMA profiles taken during the reactor trials.
- High levels of phosphate attenuation were again noted during the trials (averaging 50% and up to 80% P removal). The data from this trial suggests a biological, rather than a purely physical, basis for phosphate attenuation in the system.

The results presented in this section suggested that a modified reactor configuration, with an enhanced AF section allowing greater biomass retention and biofilm formation, benefited the performance and efficiency of LTAD of synthetic sewage. The results of a trial to evaluate further the potential of the bioreactor, using real rather than synthetic sewage wastewater, are presented in Section 4.

4 Low Temperature (12°C) Anaerobic Treatment of Sewage in Expanded Granular Sludge Bed Hybrid and Anaerobic Membrane Bioreactors

The data obtained from the trials described in Sections 2 and 3 suggested that the anaerobic treatment of sewage could be feasible under Irish conditions. The use of a synthetic wastewater for those trials, however, although convenient and safe for laboratory applications, meant that the results required verification with a non-synthetic sewage influent, which would be subject to the normal fluctuations in composition and strength associated with this wastewater. This section, therefore, presents the results of an investigation as to the feasibility for direct anaerobic treatment of sewage using the novel bioreactors described in Section 3. Both raw sewage and primary effluent (following the settlement of primary sludge) from the Mutton Island Wastewater Treatment Plant at Galway city were employed for a trial of over 150 days. Bioreactor performance and sludge physiology were monitored. The hypothesis being tested was that long-term, low-temperature (12°C), anaerobic treatment of sewage could be achieved successfully, with operational performance comparable to that of the synthetic sewage trials described in Sections 2 and 3.

In addition, the potential of an additional membrane-based module for the further enhancement of effluent quality, was investigated by the integratation of a Tangential Flow Filtration Unit into the anaerobic bioreactor.

4.1 Experimental

4.1.1 Source of Biomass, Reactor Design and Operation

Two 3.5 l (active liquid volume, 2.9 l) glass laboratory-scale bioreactors were used during the present study. A granular, mesophilic, anaerobic biomass (VSS, 102.77 g l^{-1}) obtained from a full-scale, IC bioreactor, operating at 37°C, at Carberry Milk Products (Balineen, Co. Cork, Ireland) treating milk-product production process wastewaters was used as inoculum. The bioreactors (R6 and R7) were operated at 12°C, a liquid upflow velocity of 2.5 m h^{-1} and were inoculated with 20 g VSS l^{-1} of the granular seed biomass. The bioreactors were employed to treat: (i) raw sewage over 4 operational periods (R6, PI–PIV); and (ii) primary effluent over 5 operational periods (R7, P1–PV). The influents ([i] raw sewage, following screening and grit removal; and [ii] primary effluent [following screening, grit removal and primary settlement]) were procured from the Mutton Island Wastewater Treatment Plant. Phase characteristics and operational parameters are outlined below (Table 4.1).

4.1.2 Analytical Techniques

Influent, effluent and biogas were sampled routinely. Temporal biogas methane content and effluent COD removal efficiency (CODRE) were determined according to standard methods (APHA, 1998). Biogas yields were measured using a wet gas-meter (Ritter, Bochum, Germany). Volatile fatty acids (VFAs) were monitored by GC-MS. Phosphate (PO_4^{3-}) analysis was carried by spectrophotometry (HACH Lange, USA).

4.1.3 SMA Assays

The SMA of the seed biomass and bioreactor biomass was ascertained on day 86 and on takedown day 141 (R6), day 149 (R7) at 37 and 12°C as described in Section 2.

4.1.4 Microbial Community Structure

The methanogenic community structure was determined for R6 and R7 biomass at the conclusion of the trial (day 282) by 16S rRNA clone library analysis as described in Section 2.

4.2 Results and Discussion

4.2.1 R6 Process Performance

The feasibility of direct LTAD of raw sewage was investigated in bioreactor R6. During the initial phase of operation, the bioreactor adapted quickly to the influent with COD removal being recorded within 5 days (Fig. 4.1). Fluctuations in influent resulted in similar responses in effluent quality. This trend ceased

from day 16 to the completion of PI with effluent quality displaying consistent COD concentration with an average of 135 mg l^{-1}. Performance improved further during PII and PIII of operation with decreases in HRT displaying no negative effect. The mean effluent COD concentration for PII and PIII was 111 mgl^{-1} (Table 4.1) which is within the Irish wastewater treatment regulations discharge limit of 125 mg l^{-1} [S.I. No. 419/1994]. Period IV involved a decrease in HRT to 6 h which coincided with an increase in influent strength being recorded with a concurrent decrease in effluent quality. Mean effluent quality was 200 mg l^{-1}, representing >63% COD removal.

In order to evaluate the potential for LTAD to be maintained at these elevated loading rates, an external tangential flow membrane unit with an 0.1 µm pore size was integrated to the R6 bioreactor system, during PIV, resulting in significantly improved effluent quality (Fig. 4.3) to a mean COD removal efficiency of >75%. The anaerobic membrane bioreactor, thus constituted, continued to meet discharge limits even at this elevated loading rate. The overall performance of the LTAD process remained stable throughout PIV (Fig. 4.1), suggesting that a two-phase AD-membrane process could be operated stably, and at elevated loading rates, for prolonged periods.

Phosphate (PO_4^{3-}) analysis of both influent and bioreactor effluent carried out periodically suggested the occurrence of anaerobic phosphate uptake/ accumulation by the bioreactor biomass with mean phosphate attenuation of the influent >73% (Table 4.1), which is in agreement with the data from synthetic wastewater trials presented in Sections 2 and 3.

4.2.2 R7 Process Performance

Bioreactor R7 was employed to treat primary effluent. This is the liquid stream obtained following the settlement of primary sludge from the raw sewage. The characteristics of this wastewater were considerably different to the R6 influent (raw sewage). The settling-out of the majority of solids resulted in a reduced polluting potential (COD) and phosphate (PO_4^{3-}) concentration. Within 16 days of operation, the effluent of R7 was, in terms of COD concentration, meeting discharge standards and this was the case for the remainder of PI (Fig. 4.2). The changes

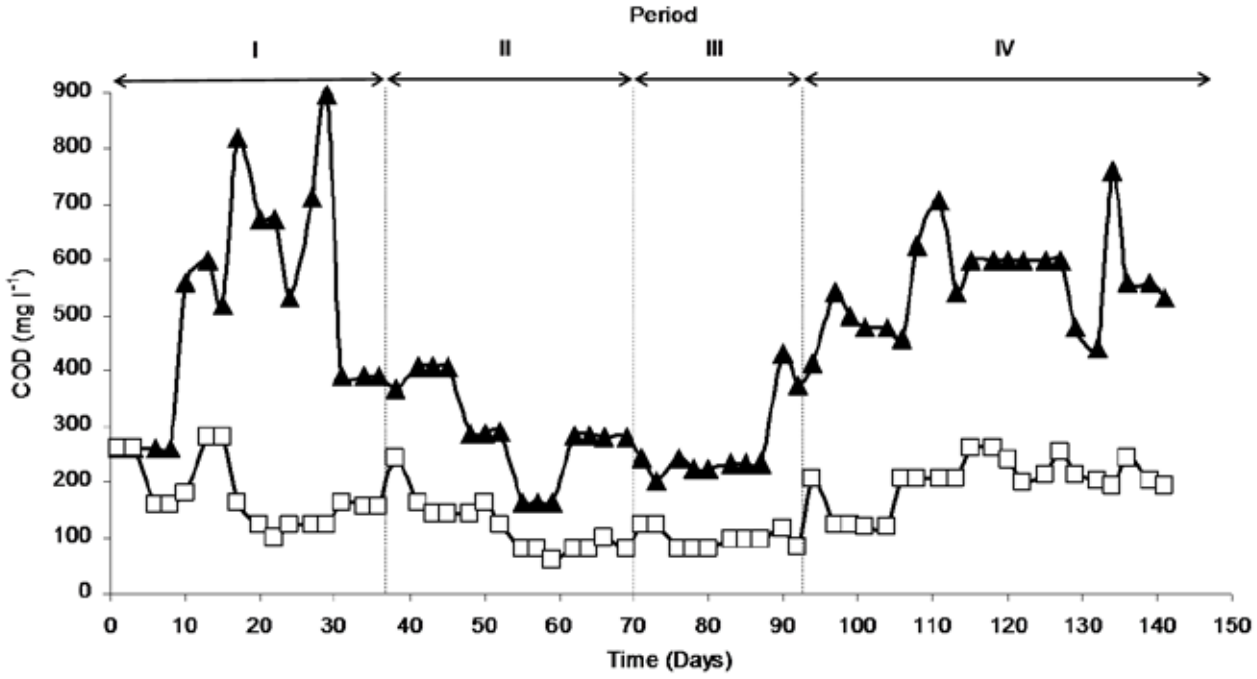


Figure 4.1. R6 chemical oxygen demand (COD) (total) concentration; influent (▲); effluent (□).

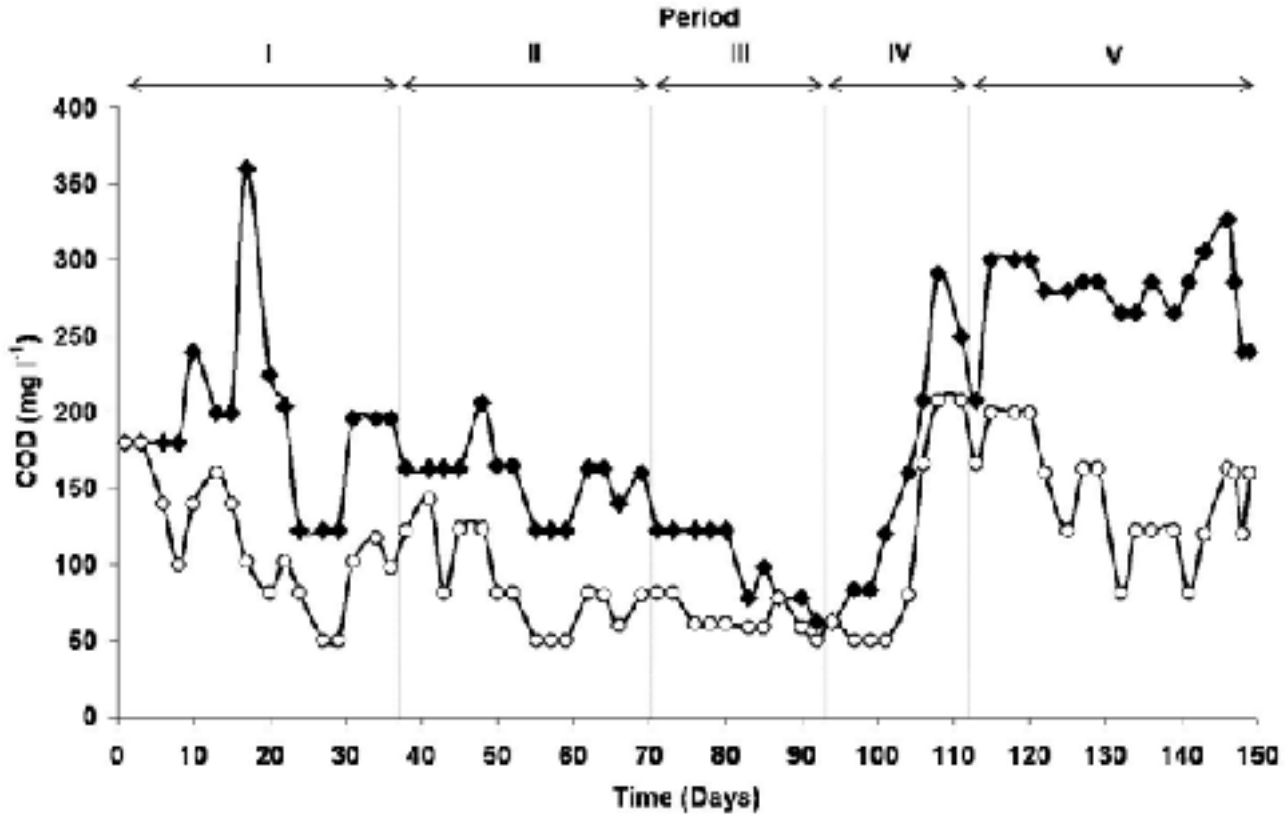


Figure 4.2. R7 chemical oxygen demand (COD) (total) concentration; influent (♦); effluent (○).

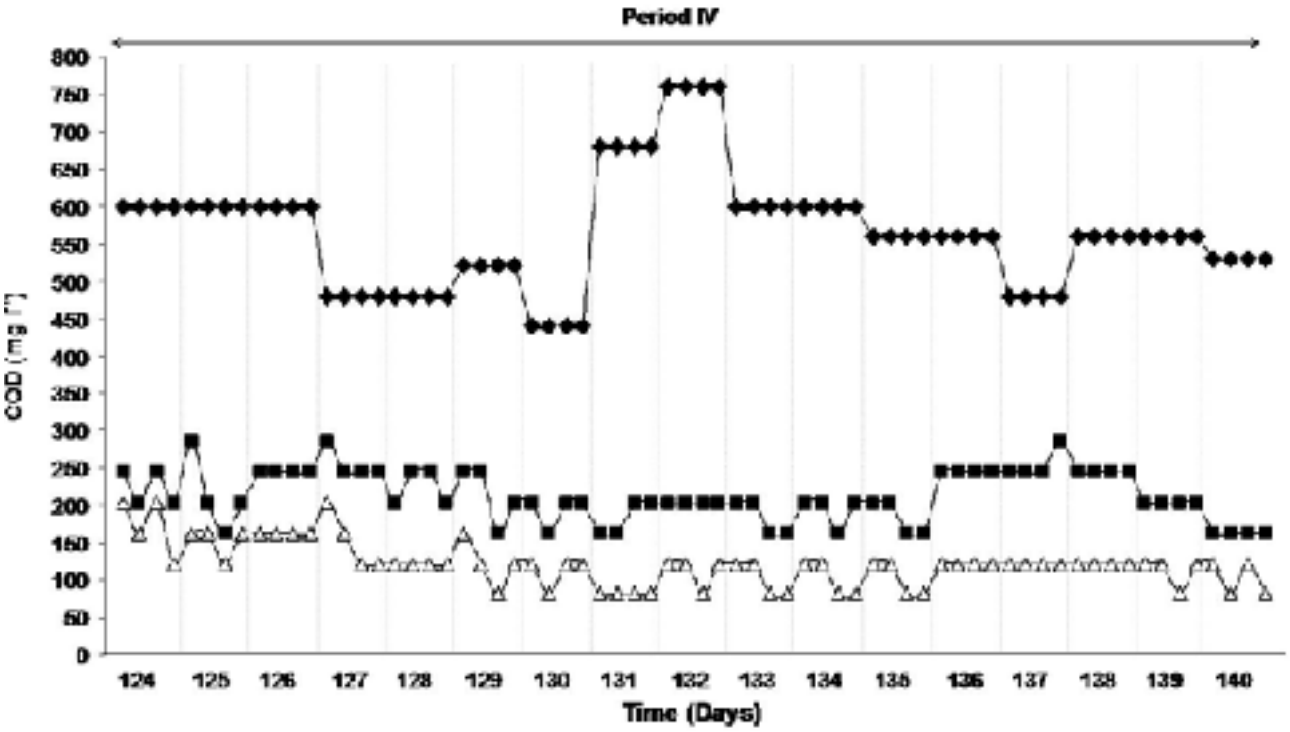


Figure 4.3. Chemical oxygen demand (COD) removal efficiency of R6 and the R6/anaerobic membrane bioreactor between days 124 and 139. Sewage influent (♦); R6 effluent (■); Membrane-filtered effluent (Δ).

Table 4.1. Operational and performance characteristics of bioreactors R6 and R7.

Bioreactor	R6				R7				
Period	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**	**V**
Days	0–37	38–70	71–93	94–141	0–37	38–70	71–93	94–112	113–149
HRT [a]	24	12	8	6	24	12	8	6	3
VLR [b]	1	2	3	4	1	2	3	4	8
SLR [c]	0.05	0.1	0.15	0.2	0.05	0.1	0.15	0.2	0.4
Influent COD Total (mg l^{-1})	512.8 ±207.2	291.4 ±86.0	265.4 ±74.6	556 ±85.2	194.1 ±55.9	155.9 ±22.6	100.8 ±24.3	157.2 ±84.9	277.9 ±27.8
Effluent COD Total mg l^{-1})	175.7 ±60.2	120.8 ±49.9	98.4 ±17.0	200.6 ±44.0	125.3 ±60.1	86.3 ±30.5	65.2 ±11.1	111.8 ±70.6	145.9 ±36.4
COD Removal Efficiency (%)	57.2	58.3	60.9	63.5	36.8	45.6	32.7	31.7	47.1
Influent phosphate (mg l^{-1} PO_4^{3-})	17.9 ±3.8	12.7 ±4.9	11.1 ±5	20.6 ±4.4	5.1 ±1.3	4.7 ±2.2	2.1 ±1.7	3.8 ±1.6	9.8 ±3.3
Effluent phosphate (mg l^{-1} PO_4^{3-})	4.1 ±1.4	4.1 ±0.9	2.1 ±0.8	4.4 ±1.9	3.5 ±2	2.9 ±1.9	0.6 ±0.6	1 ±1	4.2 ±2.4
VFA effluent (mg l^{-1} acetic acid)	0.62	0.3	0.32	1.47	0.53	0.38	0.28	0.68	1.78

All values are the phase mean ± phase standard deviation; [a] Hydraulic retention time (h); [b] Volumetric loading rate ($m^3_{Wastewater}$ $m^{-3}_{Reactor}$ d^{-1}); [c] Sludge loading rate ($m^3_{Wastewater}$ kg[VSS]$^{-1}$ d^{-1}).

to HRT during PII and III had little effect on system performance, with effluent COD concentration returning a mean over the two periods of <78 mg l^{-1} (Table 4.1). A decrease in HRT to 6 h in Period V had no immediate effect on effluent quality, with the average effluent COD concentration remaining low for the initial 10 days of PIV (59 mg l^{-1}). The increase in influent COD concentration observed with the raw sewage trial from day 90 onwards was also exhibited with the primary effluent trial. This resulted in a declining effluent quality for the latter half of PIV (mean 194 mg l^{-1}), and this decline ceased towards completion of the phase. Period V saw a drop in HRT to 3 h, and the influent COD concentration remained consistent from that recorded towards the end of the previous phase; however, as the final phase progressed, effluent quality improved, returning a mean effluent COD concentration of 146 mg l^{-1}.

4.2.3 Physiological Characterisation of Biomass

Specific methanogenic activity tests were employed to assess the activity of the various trophic groups found within the anaerobic biomass. Substrates used are intermediates of the AD process, and thus supplying each of these on an individual basis to the biomass allows the assessment of the activity of each trophic group. The SMA values obtained from the seed inoculum confirmed the mesophilic nature of the seed inoculum, with the reduced temperature of 12°C having an effect on the biomass with all substrates displaying a decrease in activity (Table 4.2). This was to be expected as the seed biomass was sourced from a full-scale bioreactor operating in a mesophilic temperature range, and was not of particular concern as the development of low-temperature activity following exposure to low-temperature conditions in bioreactors has been documented previously (Enright et al., 2005). Recorded values for the direct methanogenic precursors acetate and H_2/CO_2 at 37°C on the seed inoculum indicated the dominance of acetoclastic methanogens producing CH_4 through the decarboxylation of acetate as the primary route of methane production in favour of the reductive action of hydrogenotrophic methanogens acting on CO_2 with hydrogen. This contrasted with the recorded values of the seed inoculum tested at 12°C, which suggested the slight dominance of a hydrogenothrophic methanogenic population: however, this may be because of increased gas solubility at lower temperatures (Lettinga et al., 2001). Specific methanogenic activity assays on day 86 assessed the effect on the seed inoculum of the influent and the changes associated with the operational phases. For both the R6 and R7 biomass tested, at the bioreactor operational temperature of 12°C, increases in potential activity was observed for all substrates with the exception of propionate and butyrate where no significant change was recorded. This suggests the development of a low-temperature tolerant biomass. Increases were greatest for the R6 biomass, the more concentrated influent (raw sewage), presumably offering more abundant substrate for the various trophic groups involved in the AD process. Assays against the direct methanogenic substrates at 12°C indicated a predominantly hydrogenothrophic methanogenic community for both bioreactors. This was particularly evident in R6 where a 5-fold increase in activity from the seed inoculum analysis against H_2/CO_2 was observed. The dominance of hydrogenothrophic methanogens in low temperature bioreactors is well documented (Collins et al., 2006). Biomass from both reactors that were tested at 37°C returned greater values than those recorded at 12°C, with similar relative structures being observed. Specific methanogenic activity testing of bioreactor biomass samples at their operational temperature (12°C) on completion of the trial (R6 day 141, R7 day 149) displayed increased activity against the indirect methanogenic substrates from those observed on the previous test day, suggesting that both bioreactors contain biomass that adapts to their respective influents and also to low-temperature conditions (Table 4.2). The hydrogenothropic methanogen dominance observed on day 86 appeared to have reduced when the takedown biomass was tested, with decreases in H_2/CO_2 mediated methanogenic activity coupled with increased activity against acetate, suggesting the emergence of a more balanced methanogenic community structure. The importance of a stable acetoclastic methanogenic community to the successful operation of anaerobic digesters in terms of both methane production and granule integrity has been described previously (Lettinga, 1995, O'Flaherty et al., 1997). Once again, activity greatly increased when the biomass was tested at 37°C. The SMA assays indicate the development of a low-temperature tolerant mesophilic biomass rather than a truly low-temperature (psychrophilic) microbial community.

Table 4.2. Specific methanogenic activity (ml CH_4 g [VSS]$^{-1}$ d^{-1}) of bioreactor(s) biomass.

Day	Biomass	Test temp. (°C)	Ethanol	Acetate	Propionate	Butyrate	H_2/CO_2
0	Seed	12°C	1.7 ±0.2	2 ±0.3	1.3 ±0.1	1.8 ±0	4.6 ±0.2
	Inoculum	37°C	6.8 ±1.3	19.7 ±3.1	1.9 ±0.4	2.5 ±0.1	11.4 ±0.3
86	R6 GSB	12°C	5.1 ±0.8	5.4 ±0.7	1.1 ±0.2	1.8 ±0.1	23.9 ±0.9
		37°C	14.9 ±0.4	56.8 ±3.9	1.5 ±0.1	2.5 ±0.5	85.9 ±5.8
86	R7 GSB	12°C	3.6 ±0.1	3.3 ±0.1	1 ±0.1	1.7 ±0.1	7.7 ±0.2
		37°C	9.1 ±0.3	30.1 ±0.1	1.5 ±0.1	3.8 ±0.2	74.2 ±3.1
141	R6 GSB	12°C	7.7 ±0.5	8.3 ±0.4	1.6 ±0.3	2.9 ±0.6	7.1 ±0.5
		37°C	19.8 ±2.0	49.6 ±1.9	1.5 ±0.1	4.5 ±0.1	53.1 ±0.1
149	R7 GSB	12°C	4.3 ±0.2	4.2 ±0.3	1.2 ±0.1	1.9 ±0.1	6.3 ±0.2
		37°C	14.1 ±0.8	30.3 ±0.7	2.6 ±0.1	3.9 ±0.1	69.9 ±5.2

GSB – granular sludge bed biomass; All values are the mean of triplicates ± standard deviation.

4.2.4 Methanogenic Community Structure

Archaeal 16S rRNA gene clone library analysis of the seed sludge and R6 and R7 bioreactor samples taken at the conclusion of the reactor trials revealed that the methanogens included a high abundance of acetoclastic *Methanosaeta*-like clones (*c.* 65–75%, Fig. 4.4a–c), followed by members of the hydrogenotrophic *Methanobacteriales* group. The lack of major population shifts observed during the trial suggests that the methanogens, present in the seed inoculum, had adapted to low-temperature cultivation successfully, rather than a requirement for the emergence of specialist organisms, a fact consistent with the physiological SMA data.

Unsurprisingly, a greater level of diversity was observed in the bacterial 16S rRNA gene clone library (Fig. 4.5a–c). The most significant features of this analysis were: (i) there was no apparent loss of diversity during long-term reactor operation, a fact which is important for process stability and robustness; and (ii) the low-resolution community structure of both R6 and R7 was similar to that of the seed biomass (Fig. 4.5a–c), suggesting that a similar methanogenic consortium was present in both bioreactors.

4.3 Conclusions

- Low temperature (12°C) anaerobic digestion of raw sewage in an EGSB-AF bioreactor is entirely feasible at HRTs of 6–24 h. Effluent discharge quality in terms of COD concentration (Irish [S.I. No. 419/1994]) was consistently achieved at HRTs of 8–24 h, with concurrent methane production. The addition of a membrane-filtration unit to the EGSB-AF system for 15 days (corresponding to 60 hydraulic retention times) allowed discharge limits to be met at HRTs as low as 3 h, while stable AD was maintained.
- The rapid adaptability of the biomass to changes in HRT and influent strength suggest that operational parameters could be adjusted seasonally as required without adverse effect on performance.
- While successful treatment of primary effluent in R7 was achieved, the strength of influent being treated was low, thus offering little recompense in terms of bioenergy (methane) produced.

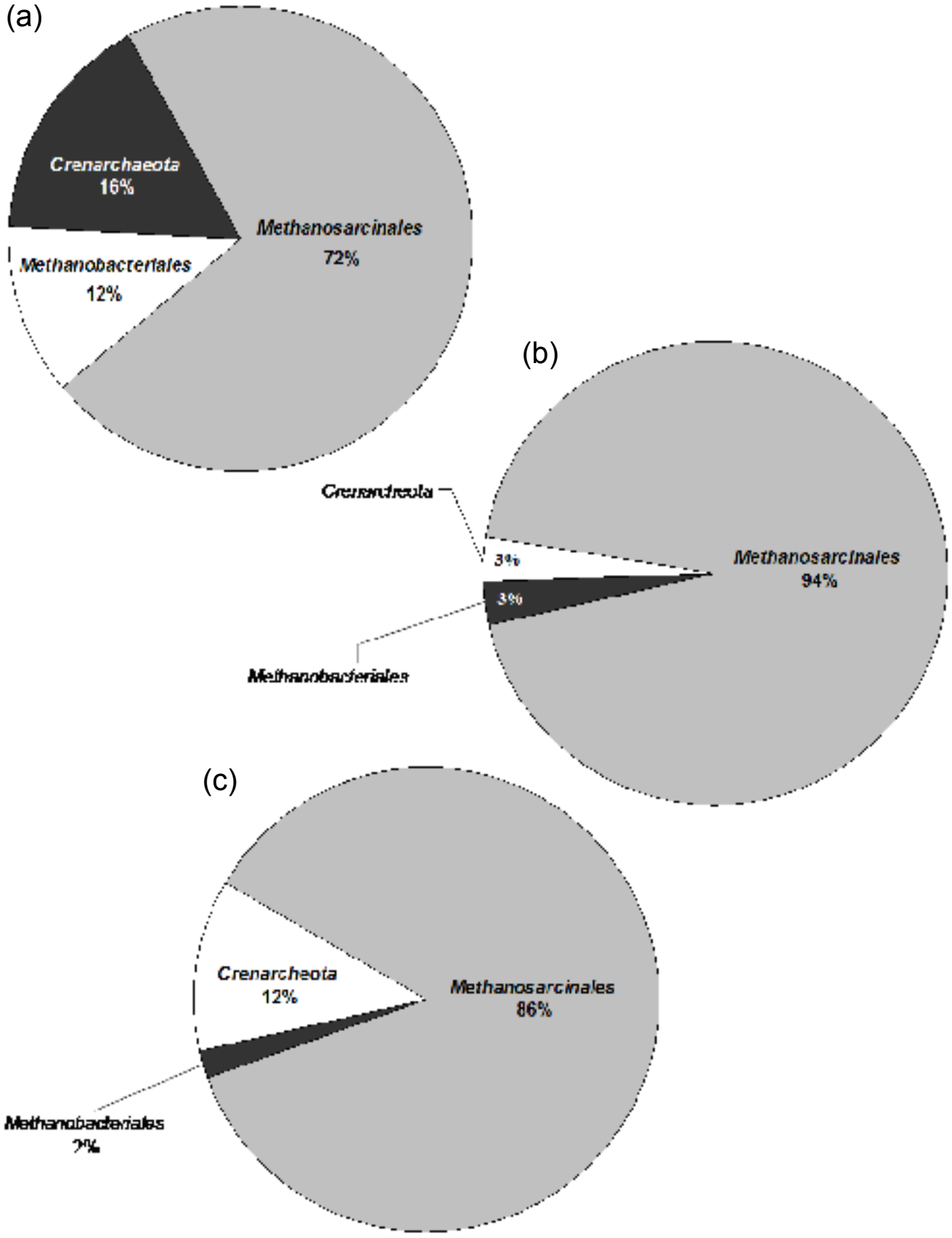


Figure 4.4. Relative abundance of Archaeal groups in 16S rRNA clone libraries from seed biomass (a) and the R6 (b) and R7 (c) bioreactors.

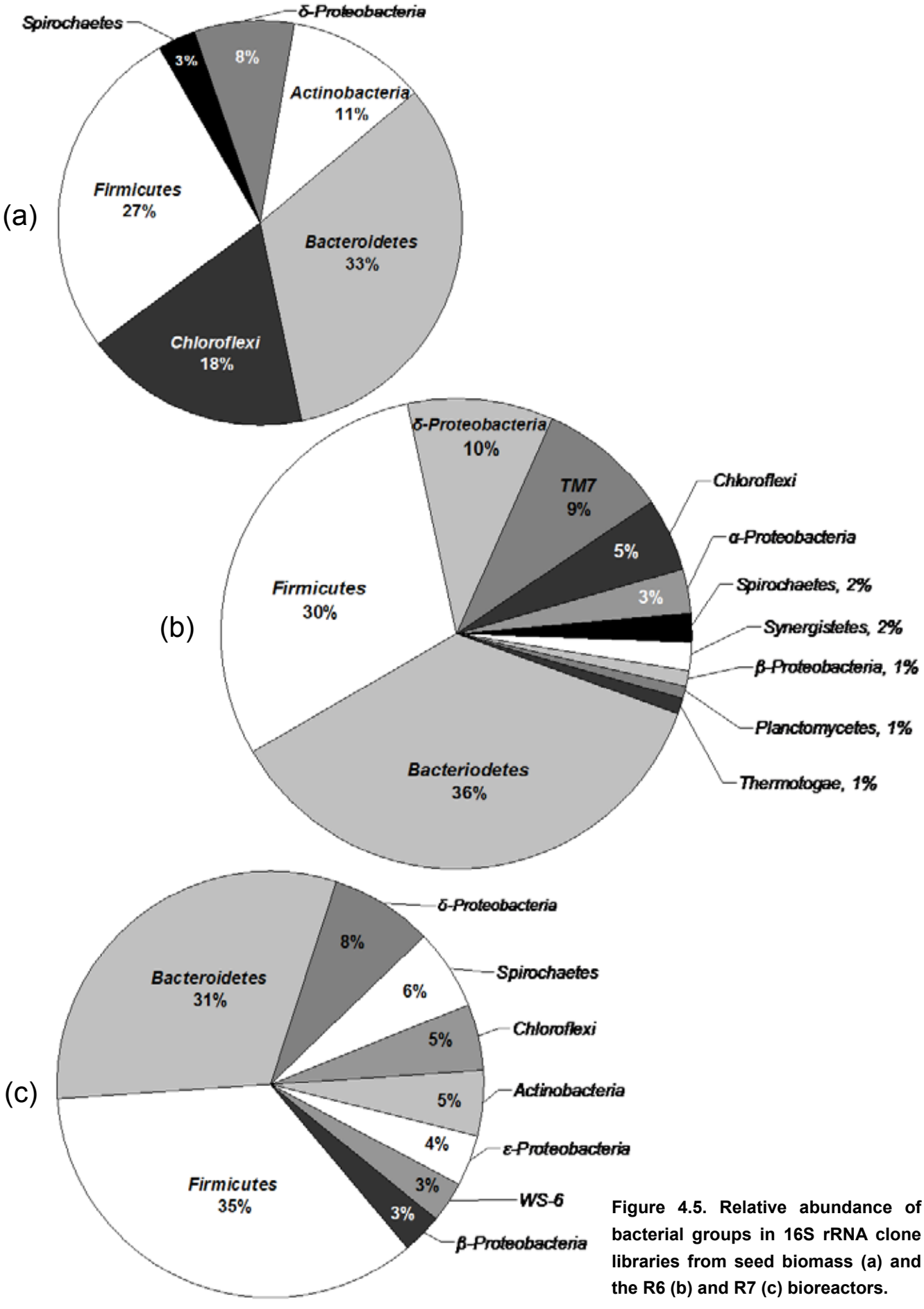


Figure 4.5. Relative abundance of bacterial groups in 16S rRNA clone libraries from seed biomass (a) and the R6 (b) and R7 (c) bioreactors.

5 Overall Project Conclusions

The calorific value of biogas is *c*. 6 kWh/m^3, which is equivalent to 0.5 l of diesel oil. The *potential* biogas yield from LTAD of sewage, based on the results of this project, ranges between 0.1 and 0.26 m^3 per person per day, depending on dilution. The energy produced, therefore, from a 1000-p.e. LTAD sewage treatment plant is the equivalent of 50–130 l of diesel per day (18–45,000 l/annum), which is available for use in the other wastewater treatment processes (drying, nutrient removal, disinfection, etc.); or for community use as vehicle fuel, household heating or power generation. In addition, and importantly, the displacement of heavy fossil fuel-utilising activated sludge plants with LTAD systems would give significant additional energy and greenhouse gas benefits (the water industry is the fourth most energy intensive sector in the UK; www.statistics.gov.uk*)*.

5.1 Process Technology

Low-temperature anaerobic digestion was shown to be effective for the high-rate treatment of both raw- and primary-settled sewage. In particular, high organic loading and short HRTs were sustainable in the bioreactors designed in NUI, Galway, for example, an OLR of 6 kg COD m^{-3} d^{-1} and an HRT of 1.5 h. The maximum OLR threshold for the laboratory-scale hybrid reactor trials was 40 kg COD m^{-3} d^{-1}, which was obtained using a concentrated synthetic wastewater (Section 2). Further work, employing pilot-scale systems using gradual OLR increments, is required to determine more precisely the precise range of operational criteria for particular wastewaters. Nevertheless, it is clear that high-rate AD treatment of sewage is feasible, with comparable performance efficiencies, OLRs and sludge-loading rates to those employed under mesophilic conditions.

It is important to state that the application of LTAD for sewage treatment will not be a single-stage full-treatment process. Rather, LTAD can act as an energy-producing core-technology in a *de novo* municipal wastewater treatment plant configuration. This will remove the great majority of organic pollutants, pathogens (Smith et al., 2005) and oxygen demand from the wastewater. Further effluent polishing (e.g. using passive systems such as constructed wetlands), nutrient removal (nitrogen and remaining phosphate) and hygenisation/disinfection may be required prior to discharge to sensitive waterbodies.

5.2 Biomass Characteristics

The research reported here has demonstrated that there are no fundamental microbiological barriers to the implementation of AD for domestic sewage treatment. A greater insight into the microbial populations involved in the AD process, at a micro-scale, will undoubtedly allow for further development and optimisation of the technology.

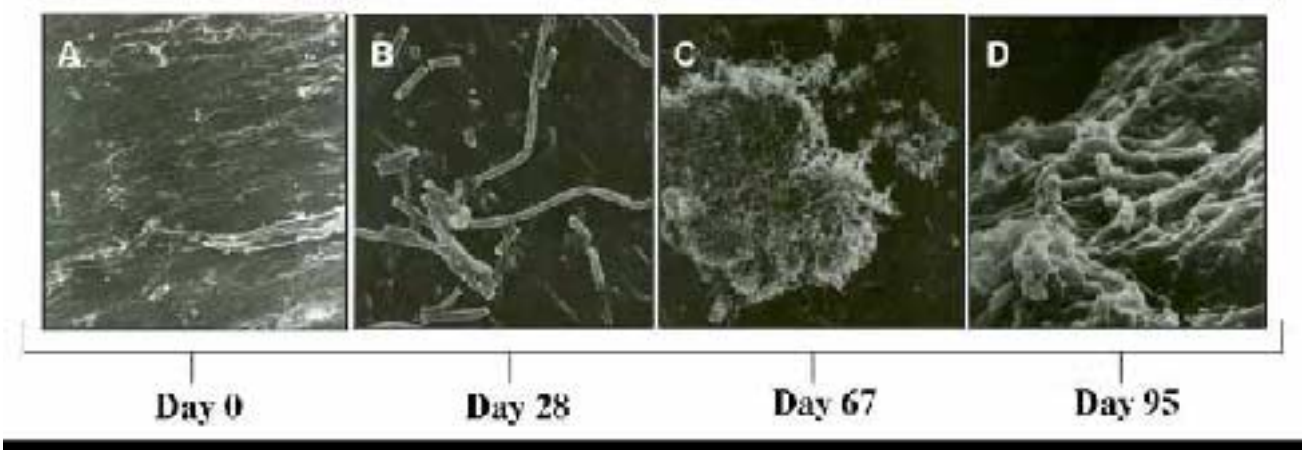


Figure 5.1. Electron micrographs illustrating the temporal microbial colonisation of carrier material under psychrophilic conditions in low-temperature anaerobic digestion (AD) bioreactors.

The formation of immobilised microbial biofilm under psychrophilic conditions was observed in all bioreactors. The upper fixed-film section of the hybrid reactors offered a 'polishing' step for the degradation of acidified wastewater from the initial upflow-bed stages.

Specific methanogenic activity collated at 12 and 15°C for the reactor sludges were higher than those recorded at 37°C for the seed sludges, revealing satisfactory development of the methanogenic and acetogenic activity of the microbial communities through low-temperature reactor cultivation. Nevertheless, the microbial populations involved in the metabolism of the substrates studied in the batch tests generally remained mesophilic (temperature optima of 37˚C), even after extended reactor operation. However, evidence of psychrophilic hydrogen-, butyrate- and propionate-catabolising communities was obtained from SMA assays in biomass from the very longest trials – it is likely that, during long-term (>1000 days) reactor operation, these organisms would emerge to significant levels in the biomass of the reactor.

Given the variable nature of anaerobic granular sludge with respect to microbial composition, physical architecture and appearance, settleability and methanogenic activity, the choice of seed biomass for wastewater treatment plants is of extreme importance. Despite the general unavailability of psychrophilically-cultivated anaerobic sludge, satisfactory LTAD was achieved using sludge from mesophilic digesters as seed biomass. The development of a specific low-temperature inoculum in the laboratory, however, resulted in a greatly reduced start-up time and more robust performance and is therefore recommended for use in future scale-up trials.

5.3 Recommendations for the Future Development of Low-temperature Anaerobic Digestion for Sewage Treatment

5.3.1 Pilot/Demonstrator Scale LTAD of Sewage

In order to progress towards the implementation of LTAD, the process must be demonstrated at a scale convincing to the water industry and policy makers. To begin the process of commercial application of LTAD, an Invention Disclosure Form was lodged with the NUI, Galway Technology Transfer Office on the novel low-temperature bioreactor designed at NUI, Galway, used in these trials. In addition, funding has been sought to construct a 150–200 p.e. mobile demonstrator-scale AD plant. A number of potential sources for funds have been explored, and the research team is hopeful of a successful outcome. The new demonstrator plant should be installed at the EPA Tuam Water Research Facility in Co. Galway, or at alternate sites in early 2011.

5.3.2 Establishing the Basis for Phosphate Removal during LTAD of Sewage

A biological, rather than a purely physical, basis for phosphate attenuation during LTAD is suggested based on data presented in Sections 2 and 3. This finding is significant and represents an additional potential advantage of the LTAD approach for sewage treatment. It is the intention of the research team to establish the basis for phosphate removal during LTAD of sewage during the pilot-scale trials planned for 2011.

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Acronyms and Annotations

AD	Anaerobic digestion
AF	Anaerobic filter
C1	Single carbon
COD	Chemical oxygen demand
CSTR	Continuously stirred tank reactor
DDW	Deionised and distilled water
EGSB	Expanded granular sludge bed
EPS	Extra-cellular polymeric substances
HRT	Hydraulic retention time
HUSB	Hydrolysis upflow sludge bed reactor
IC	Internal circulation
ICR	Internal circulation reactors
LTAD	Low-temperature anaerobic digestion
OHPA	Obligate hydrogen producing acetogens
OLR	Organic loading rates
p.e.	Person equivalent
PCR	Polymerase chain reaction
RACOD	Readily acidifiable chemical oxygen demand
SMA	Specific methanogenic activity
SRB	Sulphate-reducing bacteria
SRT	Solids retention time
UASB	Upflow anaerobic sludge blanket
VFAs	Volatile fatty acids
VSS	Volatile suspended solids

Appendix: Publication List

Hughes, D. (2011) Low-temperature anaerobic treatment of sewage. PhD Thesis, National University of Ireland, Galway

Hughes D, Enright A-M, Mahony T & O'Flaherty V (2010) Low-temperature anaerobic treatment of sewage in temperate climatic regions. In: *Proceedings of the 12th World Congress on Anaerobic Digestion*, 31 October – 4 November, Guadalahara, Mexico

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Hughes D, Enright A-M, Mahony T & O'Flaherty V (2011) Long-term (500 days), low-temperature (10–15°C), anaerobic biotreatment of raw and settled sewage: Bioprocess performance and physiological characteristics. *Water Research* (In preparation)

Hughes D, Kelly O, Enright A-M, Mahony T, Collins G and O'Flaherty V (2011) Psychrophilic methanogenic community development during long-term cultivation of anaerobic granular biofilms. *ISME Journal* (In preparation)

An Ghníomhaireacht um Chaomhnú Comhshaoil

Is í an Gníomhaireacht um Chaomhnú Comhshaoil (EPA) comhlachta reachtúil a chosnaíonn an comhshaol do mhuintir na tíre go léir. Rialaímid agus déanaimid maoirsiú ar ghníomhaíochtaí a d'fhéadfadh truailliú a chruthú murach sin. Cinntímid go bhfuil eolas cruinn ann ar threochtaí comhshaoil ionas go nglactar aon chéim is gá. Is iad na príomh-nithe a bhfuilimid gníomhach leo ná comhshaol na hÉireann a chosaint agus cinntiú go bhfuil forbairt inbhuanaithe.

Is comhlacht poiblí neamhspleách í an Ghníomhaireacht um Chaomhnú Comhshaoil (EPA) a bunaíodh i mí Iúil 1993 faoin Acht fán nGníomhaireacht um Chaomhnú Comhshaoil 1992. Ó thaobh an Rialtais, is í an Roinn Comhshaoil agus Rialtais Áitiúil a dhéanann urraíocht uirthi.

ÁR bhFREAGRACHTAÍ

CEADÚNÚ

Bíonn ceadúnais á n-eisiúint againn i gcomhair na nithe seo a leanas chun a chinntiú nach mbíonn astuithe uathu ag cur sláinte an phobail ná an comhshaol i mbaol:

- áiseanna dramhaíola (m.sh., líonadh talún, loisceoirí, stáisiúin aistrithe dramhaíola);
- gníomhaíochtaí tionsclaíocha ar scála mór (m.sh., déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta);
- diantalmhaíocht;
- úsáid faoi shrian agus scaoileadh smachtaithe Orgánach Géinathraithe (GMO);
- mór-áiseanna stórais peitreail.
- Scardadh dramhuisce

FEIDHMIÚ COMHSHAOIL NÁISIÚNTA

- Stiúradh os cionn 2,000 iniúchadh agus cigireacht de áiseanna a fuair ceadúnas ón nGníomhaireacht gach bliain.
- Maoirsiú freagrachtaí cosanta comhshaoil údarás áitiúla thar sé earnáil - aer, fuaim, dramhaíl, dramhuisce agus caighdeán uisce.
- Obair le húdaráis áitiúla agus leis na Gardaí chun stop a chur le gníomhaíocht mhídhleathach dramhaíola trí comhordú a dhéanamh ar líonra forfheidhmithe náisiúnta, díriú isteach ar chiontóirí, stiúradh fiosrúcháin agus maoirsiú leigheas na bhfadhbanna.
- An dlí a chur orthu siúd a bhriseann dlí comhshaoil agus a dhéanann dochar don chomhshaol mar thoradh ar a ngníomhaíochtaí.

MONATÓIREACHT, ANAILÍS AGUS TUAIRISCIÚ AR AN GCOMHSHAOL

- Monatóireacht ar chaighdeán aeir agus caighdeáin aibhneacha, locha, uiscí taoide agus uiscí talaimh; leibhéil agus sruth aibhneacha a thomhas.
- Tuairisciú neamhspleách chun cabhrú le rialtais náisiúnta agus áitiúla cinntí a dhéanamh.

RIALÚ ASTUITHE GÁIS CEAPTHA TEASA NA HÉIREANN

- Cainníochtú astuithe gáis ceaptha teasa na hÉireann i gcomhthéacs ár dtiomantas Kyoto.
- Cur i bhfeidhm na Treorach um Thrádáil Astuithe, a bhfuil baint aige le hos cionn 100 cuideachta atá ina mór-ghineadóirí dé-ocsaíd charbóin in Éirinn.

TAIGHDE AGUS FORBAIRT COMHSHAOIL

- Taighde ar shaincheisteanna comhshaoil a chomhordú (cosúil le caighdéan aeir agus uisce, athrú aeráide, bithéagsúlacht, teicneolaíochtaí comhshaoil).

MEASÚNÚ STRAITÉISEACH COMHSHAOIL

- Ag déanamh measúnú ar thionchar phleananna agus chláracha ar chomhshaol na hÉireann (cosúil le pleananna bainistíochta dramhaíola agus forbartha).

PLEANÁIL, OIDEACHAS AGUS TREOIR CHOMHSHAOIL

- Treoir a thabhairt don phobal agus do thionscal ar cheisteanna comhshaoil éagsúla (m.sh., iarratais ar cheadúnais, seachaint dramhaíola agus rialacháin chomhshaoil).
- Eolas níos fearr ar an gcomhshaol a scaipeadh (trí cláracha teilifíse comhshaoil agus pacáistí acmhainne do bhunscoileanna agus do mheánscoileanna).

BAINISTÍOCHT DRAMHAÍOLA FHORGHNÍOMHACH

- Cur chun cinn seachaint agus laghdú dramhaíola trí chomhordú An Chláir Náisiúnta um Chosc Dramhaíola, lena n-áirítear cur i bhfeidhm na dTionscnamh Freagrachta Táirgeoirí.
- Cur i bhfeidhm Rialachán ar nós na treoracha maidir le Trealamh Leictreach agus Leictreonach Caite agus le Srianadh Substaintí Guaiseacha agus substaintí a dhéanann ídiú ar an gcrios ózóin.
- Plean Náisiúnta Bainistíochta um Dramhaíl Ghuaiseach a fhorbairt chun dramhaíl ghuaiseach a sheachaint agus a bhainistiú.

STRUCHTÚR NA GNÍOMHAIREACHTA

Bunaíodh an Ghníomhaireacht i 1993 chun comhshaol na hÉireann a chosaint. Tá an eagraíocht á bhainistiú ag Bord lánaimseartha, ar a bhfuil Príomhstiúrthóir agus ceithre Stiúrthóir.

Tá obair na Gníomhaireachta ar siúl trí ceithre Oifig:

- An Oifig Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig um Fhorfheidhmiúchán Comhshaoil
- An Oifig um Measúnacht Comhshaoil
- An Oifig Cumarsáide agus Seirbhísí Corparáide

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag ball air agus tagann siad le chéile cúpla uair in aghaidh na bliana le plé a dhéanamh ar cheisteanna ar ábhar imní iad agus le comhairle a thabhairt don Bhord.

NDP
National Development Plan 2007 - 2013

Science, Technology, Research and Innovation for the Environment (STRIVE) 2007-2013

The Science, Technology, Research and Innovation for the Environment (STRIVE) programme covers the period 2007 to 2013.

The programme comprises three key measures: Sustainable Development, Cleaner Production and Environmental Technologies, and A Healthy Environment; together with two supporting measures: EPA Environmental Research Centre (ERC) and Capacity & Capability Building. The seven principal thematic areas for the programme are Climate Change; Waste, Resource Management and Chemicals; Water Quality and the Aquatic Environment; Air Quality, Atmospheric Deposition and Noise; Impacts on Biodiversity; Soils and Land-use; and Socio-economic Considerations. In addition, other emerging issues will be addressed as the need arises.

The funding for the programme (approximately €100 million) comes from the Environmental Research Sub-Programme of the National Development Plan (NDP), the Inter-Departmental Committee for the Strategy for Science, Technology and Innovation (IDC-SSTI); and EPA core funding and co-funding by economic sectors.

The EPA has a statutory role to co-ordinate environmental research in Ireland and is organising and administering the STRIVE programme on behalf of the Department of the Environment, Heritage and Local Government.

epa
Environmental Protection Agency
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