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# Energy and Economic Implications of Anaerobic Digestion Pasteurisation Regulations in Ireland

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## Abstract

The use of anaerobic digestion for the treatment of organic wastes is spreading throughout Europe. A number of restrictions on organic wastes which can be treated in anaerobic digestion facilities and the subsequent handling of the digested material are specified in European legislation. Regulation 1774/2002/EC as amended states that after reduction the material must be heated to either 70 °C or 90 °C for a minimum of 60 min. An alternative Irish national standard of 60 °C for 48 h twice has been introduced in place of the EU standard. Anaerobic digestion systems are successful only if they produce a significant energy output. The aim of this research was therefore to examine both the EU and Irish national standards as well as a number of alternative treatment scenarios to determine their respective pasteurisation efficiency and energetic requirement. Post-digestion pasteurisation above 60 °C was found to satisfactorily remove all viable *E. coli* bacteria from the test feedstock. It was determined that the most energy and economically efficient heat treatments were 60 °C for 1 h, 70 °C for 1 h (EU standard), and 80 °C for 30 min. The Irish national standard was found to be prohibitively energy inefficient and expensive.

**Keywords:** Anaerobic digestion; Pasteurisation; EU legislation; Energy balance; *E. coli*.

## 1. Introduction

Anaerobic digestion is a proven technology to treat organic waste and produce methane which can be used for heat and/or electricity generation and a nutrient-rich fertiliser product which can be applied to agricultural land [1]. The use of anaerobic digestion for treatment of various

organic wastes is spreading throughout Europe as it offers an opportunity to use organic wastes as a substitute for fossil fuel usage [2]. European legislation (Regulation 1774/2002/EC, as amended) specifies a number of restrictions on the organic wastes which are suitable for treatment in anaerobic digestion facilities and the subsequent handling of the digested material [3]. For example, due to the operating temperatures of AD facilities Category 1 material (which includes animal tissue suspected of being or confirmed to be infected with a transmissible spongiform encephalopathy) is unsuitable for treatment in such a facility. Category 2 material (which includes manure and digestive tract content) and Category 3 material (which includes animal tissue fit for human consumption) can be treated in an AD facility, however [3].

Pasteurisation of the organic material is an essential biosecurity step [4] to protect both human and animal health and to reduce the spread of disease associated with bad practice when handling animal by-products. To this end, both European and national regulatory measures have been introduced: Regulation 1774/2002/EC specifies that after reduction the material must be heated to either 70 °C or 90 °C for a minimum of 60 min. This is considered the EU standard for pasteurisation to prevent the spread of pathogens or parasites in the digested material. Irish legislation also attempts to reduce the spread of disease by introducing alternative pasteurisation standards: the national processing standard of heating to 60 °C for 48 h twice; or an operator's own pasteurisation standard, provided it achieves all conditions laid out by EU/national legislation and has been validated [5].

To validate an alternative pasteurisation standard a rigorous protocol must be followed to certify that the process adequately reduces the biological risk. The standard must be able to identify all hazards associated with the system and be able to measure the reduction of pathogens in the material by using an endogenous indicator organism. The pathogens specified under Irish legislation which must be reduced are either *Enterococcus faecalis* or *Salmonella senftenberg* (a 5 log<sub>10</sub> reduction) and thermo-resistant viruses e.g. Parvovirus (a 3 log<sub>10</sub> reduction). Furthermore the pasteurisation process must also induce a 99.9% reduction in parasite eggs e.g. *Ascaris* spp. (Table 1) [5].

Table 1: Pasteurisation efficiency standards (Department of Agriculture Fisheries and Food (2009))

Indicator organism	<i>n</i>	Test parameter <sup>a</sup>		
		<i>m</i>	<i>M</i>	<i>c</i>
<i>E. coli</i> / Enterococaceae				
Digestate for own use	5	1000	5000	1
Digestate to be marketed	5	0	1000	1
<i>Salmonella</i> (post-storage sampling)				
Digestate for own use	5	0	0	0
Digestate to be marketed	5	0	0	0

<sup>a</sup>*n*: number of samples that must be tested

*m*: threshold value of bacteria (CFUs g<sup>-1</sup>) considered satisfactory provided *m* is not exceeded in all samples

*M*: maximum number of CFUs g<sup>-1</sup>; result is considered unsatisfactory if *M* is exceeded in one or more samples

*c*: number of tests permitted to be between *m* and *M*, provided the bacterial count of all other samples is *m* or less

Anaerobic digestion can take place under either mesophilic (20-45 °C) or thermophilic (50-65 °C) conditions [6]. The optimum operating temperature, considering both the potential biogas yield from digestion and the energy requirement to heat the digester, is one of the most critical factors for economically viable operation in temperate countries since most annual temperatures are below the mesophilic condition [7]. Anaerobic digestion systems are successful only if they produce a significant energy output and operational processes such as pasteurisation which has a high associated energy demand significantly influence the energy balance of the system [1]. The aim of this work was therefore to determine the pasteurisation efficiency and energetic demand of the EU and Irish national standards which have been introduced to ensure pasteurisation of AD digestate to protect human and animal health. Furthermore, a number of alternative heat treatment scenarios were examined to investigate their pasteurisation efficiencies and energetic requirements with the aim of reducing the energy and economic demands associated with pasteurisation, thereby optimising the energy output of anaerobic digestion of waste material. Neither the initial capital costs associated with anaerobic digestion, which are known to be considerable [8], nor the economical feasibility of AD facilities in Ireland are analysed in this study as a comprehensive review on this area has recently been undertaken by Goulding and Power [9].

## 2. Method and Materials

### 2.1 Pasteurisation efficacy

The EU and the Irish national pasteurisation standards use indicator organisms to validate the sterility of digested material prior to their release for application to soil. In this vein, *E. coli* O157:H7 was used in this study as an indicator organism to validate the pasteurisation efficacy of the EU and Irish national standard as well as a number of alternative heat treatments. Akbulut [10] reported higher levels of pathogen removal at higher temperatures. As such, a number of heat treatments were investigated: 40 °C for 1 h; 50 °C for 1 h; 60 °C for 1 h; 60 °C for 24 h; 60 °C for 48 h (the Irish national standard requires that these conditions must be met twice); 70 °C for 1 h (EU standard); and 80 °C for 30 min. Positive and negative controls were also included to ensure the indicator organism was viable and that contamination did not occur during the investigation.

The experimental procedure used in this research is based on the methodology of Ziembra and Peccia [11]. A feedstock for anaerobic digestion was simulated by combining dairy cow slurry (28 ml) and blended vegetable matter (12 ml). The feedstock was autoclaved at 121 °C for 20 min to sterilise the sample prior to inoculation. 1 ml of the feedstock was then aseptically added to urea broth tubes and inoculated with 1 ml of *E. coli* bacterial stock and mixed for 1 min using a vortex mixer. The test tubes were then placed in a water bath at the appropriate temperature for the appropriate length of time. Throughout the heat treatment the test tubes were periodically returned to the vortex mixer to ensure good mixing of stock. Samples were mixed for a final time at the end of the heat treatment before a seven scale serial dilution of 1:10 was carried out aseptically and duplicate 0.1 ml aliquots of the dilutions were transferred to plate count agar. The plates were incubated for 48 h at 30 °C before the colonies were counted and the number of bacteria remaining after each heat treatment was calculated.

### 2.2 Energy requirement analysis

The energy input associated with each heat treatment process was calculated according to the equations used by Salter and Banks [12]. The energy analysis investigated the energetic demands of pasteurisation at 60 °C for 1 h; 60 °C for 24 h; 60 °C for 48 h; 70 °C for 1 h; and 80 °C

for 30 min. This analysis was conducted assuming an undigested feedstock temperature of 12 °C (average daily temperature in Ireland [13]) and a digestate temperature of 40 °C (mesophilic operating temperature of the digester). This facilitated a comparison of the energy input associated with pasteurising the organic material before and after digestion.

The energy requirements of pasteurisation were calculated using operational data relating to an anaerobic digester located in the south west of the Republic of Ireland. This digester treats 10,760 tonnes of feedstock every year consisting of cattle slurry, food waste, poultry litter, dairy sludge, and glycerine. The facility operates at 40 °C and 17.15 m<sup>3</sup> of feedstock are loaded daily for digestion. The pasteurisation unit has a cross sectional area of 6.76 m<sup>2</sup>. A number of assumptions were made which relate to the digestion process: it was assumed that the specific heat capacity of the digestate was 4.18 kJ kg<sup>-1</sup> °C<sup>-1</sup>; that the digester coefficient of heat transfer was 2.0 W m<sup>-2</sup> °C; and that the digester was operating at 70% efficiency [14].

The energy demand for pasteurisation depends on the operational temperature of the digester. Digestion at thermophilic temperatures often produces more methane [15] and has been reported to have greater pathogen-kill efficiency [11] than mesophilic operations, but has a higher overall energy requirement to maintain the higher operational temperature [15], and therefore potentially a greater economic demand. As such, the energy balance for pasteurisation occurring before and after digestion occurring at mesophilic and thermophilic temperatures was evaluated. The heat required for each of the pasteurisation processes, taking into consideration heat loss through the walls of the digester [16], was calculated according to equations 1, 2, and 3 (after Salter and Banks [12]):

$$hl = UA\Delta T \quad (\text{Eq. 1})$$

$$q = CQ\Delta T \quad (\text{Eq. 2})$$

$$\text{Total heat required} = hl + q \quad (\text{Eq. 3})$$

where hl = heat loss,

U = coefficient of heat transfer (W m<sup>-2</sup> °C),

A = cross sectional area through which heat loss occurs (m<sup>2</sup>),

ΔT = temperature drop across the surface (°C),

q = the heat required to raise the temperature of the feedstock to that of the digester,

C = specific heat of the feedstock (kJ kg<sup>-1</sup> °C<sup>-1</sup>), and

Q = volume of feedstock (m<sup>3</sup>).

The economic demand associated with achieving pasteurisation temperatures is related to the temperature of the feedstock (if pasteurisation occurs before digestion) or of the digestate (if pasteurisation occurs after digestion) and the energy input needed to raise the temperature accordingly. The economic demand of each pasteurisation treatment was calculated according to most recent information available detailing the cost of energy for businesses in Ireland [17]: the annual cost of heating was calculated at €0.1017 kWh<sup>-1</sup>.

### 3. Results

It was determined that the *E. coli* stock had an initial colony count of  $4.8 \times 10^8$  CFUs g<sup>-1</sup>. This was verified by the positive control test which returned a colony count of  $4.7 \times 10^8$  CFUs g<sup>-1</sup>. The pasteurisation effect of each heat treatment is shown in Figure 1 where it can be seen that at a dilution of 1:10,000,000 there were no viable CFUs present in samples which were incubated at temperatures higher than 60 °C. It can also be seen that heating the feedstock to 40 °C for 1 h falls a long way short of adequately pasteurising the sample, reducing the bacterial count from  $4.8 \times 10^8$  CFUs g<sup>-1</sup> to  $4.2 \times 10^8$  CFUs g<sup>-1</sup>, while heating the feedstock to 50 °C for 1 h significantly reduced the number of CFUs in the sample but not to an extent satisfactory for either the EU or Irish national standard (Figure 1).

The overall energy requirements of each heat treatment which satisfied the pasteurisation requirements for *E. coli* are shown in Table 2. The energy input associated with pasteurising an undigested feedstock prior to digestion (i.e. feedstock at 12 °C) was calculated to be 2,344,981.85 kWh for the EU standard and 186,304,764.7 kWh for the Irish national standard. If pasteurisation takes place after digestion (i.e. digestate at 40 °C) the associated energy input for the EU and Irish national standards are 1,212,921.65 kWh and 77,626,985.28 kWh, respectively. Figure 2 indicates the contrast between the energy requirements to pasteurise undigested feedstock (12 °C) and digestate (40 °C).

### 4. Discussion

Abbasi, Tauseef [18] described pasteurisation of AD sludge at 70° C as an effective alternative to sterilisation at 130 °C. In this study, all of the treatments which heated the feedstock to 60° C or higher satisfied the bacterial reductions specified for *E. coli* in EU legislation, irrespective of

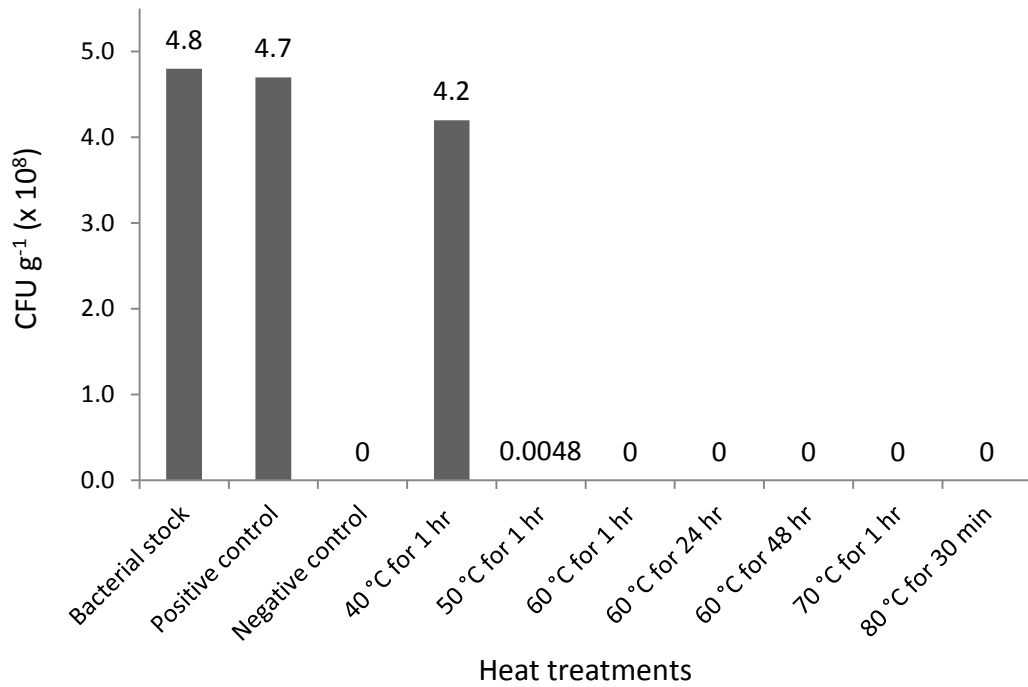


Figure 1: Results of various heat treatment processes on bacteria numbers

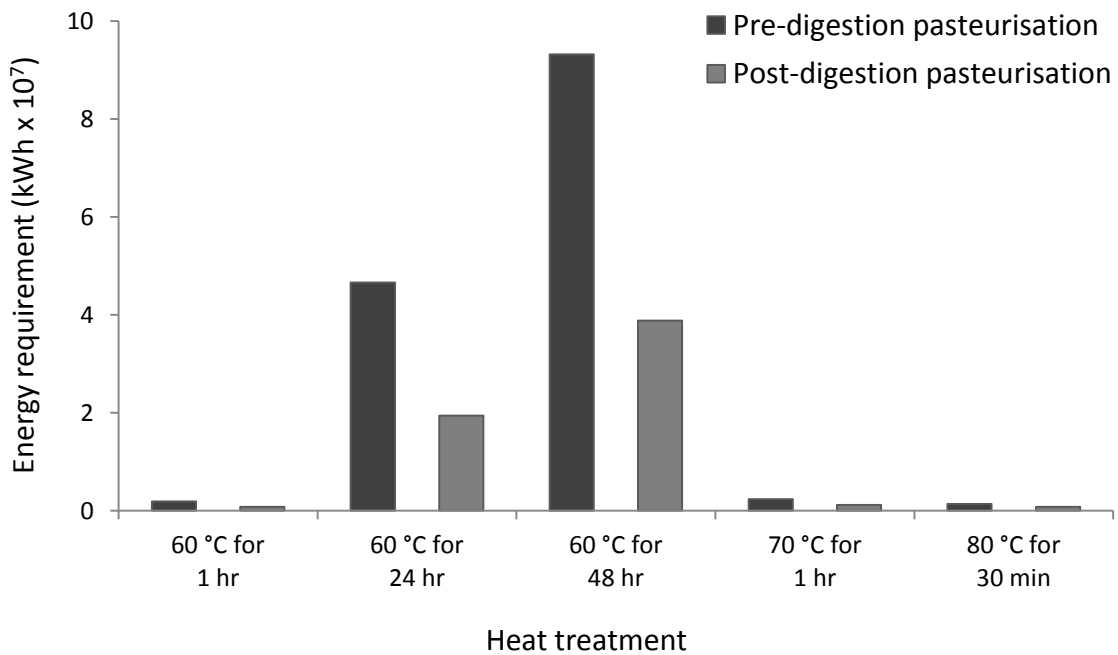


Figure 2: Energy requirements for pre- and post-digestion pasteurisation

the amount of time the organic material was held at each temperature. These results are in agreement with those of Astals, Venegas [19] who reported no detection of viable *E. coli* after post-AD treatment at 60 and 80 °C. In addition, in an evaluation of autothermal thermophilic anaerobic digestion Lloret, Pastor [20] investigated pasteurisation efficacy of digestion process in which the temperature within the digester rises to 65 °C. Under these circumstances the authors reported die off rate for *E. coli* from  $3.4 \times \log 10^6$  to absent as well as the complete absence of *Salmonella* spp. in the digestate [20]. These results suggest that there is no necessity to follow heat treatments at the EU-suggested parameters (70 °C for 1 h) or the Irish national standard (60 °C for 48 h, twice) to achieve *E. coli* removal.

The Irish national standard has a much higher energy requirement than the EU standard whether pasteurisation takes place before or after digestion. The digester used in this evaluation has an energy output of  $4.1 \times 10^6$  kWh annually. The EU and Irish national standards would therefore use 57.19% and 4,544.02% of the digester's output if the feedstock was pasteurised prior to digestion. If pasteurisation were to take place after digestion when the digestate was at 40 °C the EU and national standards would consume 29.58 and 1,893.34% of the digester's annual energy output. Put into this context, the Irish national standard has a prohibitively high energy requirement to achieve satisfactory pasteurisation.

If pasteurisation were to take place before digestion the other heat treatments which were deemed to satisfy pasteurisation requirements for *E. coli* (60 °C for 1 h and 80 °C for 30 min) would use 47.33 and 33.53% of the digester's annual energy output, respectively. Post-digestion pasteurisation at 60 °C for 1 h or 80 °C for 30 min would each consume 19.72% of the annual energy output. It can therefore be concluded that pasteurisation which occurs post-digestion is more energy efficient, and that the EU standard of 70 °C for 1 h or either of two alternative heat treatments (60 °C for 1 h or 80 °C for 30 min) are the most efficient heat treatments which successfully remove the health risks associated with *E. coli* from the digested material.

From a financial point of view post-digestion pasteurisation is a more economical option to sufficiently remove *E. coli* from the organic material (Table 2). The EU standard for sterilisation

Table 2: Energy input requirements and financial cost of each heat treatment examined

Treatment	Heating (kWh)	Heat Loss (kWh)	Heat Loss + Heating (kWh)	Heat Loss + Heating* Efficiency (kWh)	Annual Heating (kWh)	Cost of Annual Heating (at €0.1017 kWh <sup>-1</sup> ) [17]
<u>Feedstock at 12 °C</u>						
60 °C for 1 hr	3440.98	648.96	4089.94	5316.92	1940674.63	197366.61
60 °C for 24 hr	82583.42	15575.04	98158.46	127606.00	46576191.17	4736798.64
60 °C for 48 hr	165166.85	31150.08	196316.93	255212.01	93152382.34	9473597.28
70 °C for 1 hr	4157.85	784.16	4942.01	6424.61	2344981.85	238484.65
80 °C for 30 min	2437.36	459.68	2897.04	3766.15	1374644.53	139801.35
<u>Digestate at 40 °C</u>						
60 °C for 1 hr	1433.74	270.40	1704.14	2215.38	808614.43	82236.09
60 °C for 24 hr	34409.76	6489.60	40899.36	53169.17	19406746.32	1973666.10
60 °C for 48 hr	68819.52	12979.20	81798.72	106338.34	38813492.64	3947332.20
70 °C for 1 hr	2150.61	405.60	2556.21	3323.07	1212921.65	123354.13
80 °C for 30 min	1433.74	270.40	1704.14	2215.38	808614.43	82236.09

is a more favourable option than the Irish standard: the cost associated with heating the undigested organic material twice to 60 °C for 48 h (the Irish standard) is an 80 fold increase on the cost of heating the organic material to 70 °C for 1 h (the EU standard). Post-digestion pasteurisation using the Irish national standard is an almost 65 fold increase on the cost to pasteurise to the EU standard. Pre-digestion pasteurisation of the organic material using the other heat treatments which were deemed to satisfy pasteurisation requirements for *E. coli* (60°C for 1 h and 80 °C for 30 min) would cost approximately 20% and 40% less than the EU standard, respectively; post-digestion pasteurisation using the same treatments would cost approximately 33% less than the EU standard.

The heat treatments investigated here do not consider pasteurisation efficiency for the other pathogens and parasites specified by the Department of Agriculture, Fisheries and Food [5], namely *Enterococcus faecalis*, *Salmonella senftenberg* (775W, H<sub>2</sub>S negative), Parvovirus, or *Ascaris* spp. Maya, Torner-Morales [21], for example, investigated inactivation of *Ascaris* spp. in sludge and reported that temperature had an effect on inactivation provided the temperature exceeded 70 °C for more than 2 h. Work is currently ongoing to determine whether the various heat treatments which were deemed satisfactory for *E. coli* are also satisfactory for each of the

other specified organisms, particularly in light of the results reported by Maya, Torner-Morales [21].

## **5. Conclusions**

It can be concluded from this research that the risk associated with viable *E. coli* can be successfully removed from organic material by heating the undigested feedstock/digestate to 60 °C for 1 h, 24 h, or 48 h, to 70 °C for 1 h, or to 80 °C for 30 min. The heat treatments investigated here, however, do not consider pasteurisation efficiency for all of the indicator organisms specified by the Department of Agriculture, Fisheries and Food [5].

From an energy use point of view it is more efficient to pasteurise the organic material after digestion when the feedstock temperature has been raised by the digestion process. Heating the digestate to 60 °C for 1 h, to 70 °C for 1 h (the EU standard), and to 80 °C for 30 min were shown to be the most energy efficient heat treatments.

From a financial viewpoint, the EU pasteurisation standard is a more economical treatment option than the Irish national standard, which is prohibitively expensive. Heating the digestate to 60 °C for 1 h or to 80 °C for 30 min offers further financial savings over the EU standard, with post-digestion pasteurisation being the more economical option.

## **6. References**

- [1] Banks CJ, Chesshire M, Heaven S, Arnold R. Anaerobic digestion of source-segregated domestic food waste: Performance assessment by mass and energy balance. *Bioresource Technology*. 2011;102(2):612-20.
- [2] Gong W-J, Liang H, Li W-Z, Wang Z-Z. Selection and evaluation of biofilm carrier in anaerobic digestion treatment of cattle manure. *Energy*. 2011;36(5):3572-8.
- [3] European Parliament and the Council of the European Union. Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 Laying Down Health Rules Concerning Animal By-Products not Intended for Human Consumption. Brussels, Belgium: European Parliament and the Council of the European Union; 2002.

- [4] Banks CJ, Salter AM, Heaven S, Riley K. Energetic and environmental benefits of co-digestion of food waste and cattle slurry: A preliminary assessment. *Resources, Conservation and Recycling*. 2011;56(1):71-9.
- [5] Department of Agriculture Fisheries and Food. Conditions for Approval and Operation of Biogas Plants Treating Animal By-Products in Ireland. Dublin, Ireland: Department of Agriculture, Fisheries and Food Animal By-Products Section; 2009.
- [6] Monnet F. An Introduction to Anaerobic Digestion of Organic Wastes: A Final Report. Glasgow, UK: Remade Scotland; 2003.
- [7] Chae KJ, Jang A, Yim SK, Kim IS. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. *Bioresource Technology*. 2008;99(1):1-6.
- [8] Kythreotou N, Tassou SA, Florides G. An assessment of the biomass potential of Cyprus for energy production. *Energy*. 2012;47(1):253-61.
- [9] Goulding D, Power N. Which is the preferable biogas utilisation technology for anaerobic digestion of agricultural crops in Ireland: Biogas to CHP or biomethane as a transport fuel? *Renewable Energy*. 2013;53(0):121-31.
- [10] Akbulut A. Techno-economic analysis of electricity and heat generation from farm-scale biogas plant: Çiçekdağı case study. *Energy*. 2012;44(1):381-90.
- [11] Ziemba C, Peccia J. Net energy production associated with pathogen inactivation during mesophilic and thermophilic anaerobic digestion of sewage sludge. *Water Research*. 2011;45(16):4758-68.
- [12] Salter A, Banks C. Anaerobic digestion: overall energy balances – parasitic inputs & beneficial outputs. *Advances in Biological Processes for Organics and Energy Recycling*. Birmingham, UK: Sustainable Organic Resources Partnership; 2008.
- [13] Met Eireann. 30 Year Averages. [www.met.ie/climate-ireland/30year-averages.asp](http://www.met.ie/climate-ireland/30year-averages.asp) 2012. [Last accessed 19th October 2012].

- [14] Energy Systems Research Unit. Case Study: Investigated Routes of Disposal. [www.esru.strath.ac.uk/EandE/Web\\_sites/97-8/energy\\_from\\_waste/page6.htm](http://www.esru.strath.ac.uk/EandE/Web_sites/97-8/energy_from_waste/page6.htm) 1998. [Last accessed 14th November 2012].
- [15] Ward AJ, Hobbs PJ, Holliman PJ, Jones DL. Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology*. 2008;99(17):7928-40.
- [16] González-González A, Cuadros F, Ruiz-Celma A, López-Rodríguez F. Energy-environmental benefits and economic feasibility of anaerobic codigestion of Iberian pig slaughterhouse and tomato industry wastes in Extremadura (Spain). *Bioresource Technology*. 2013;136(0):109-16.
- [17] Howley M, Holland M, Dennehy E. Electricity & Gas Prices in Ireland - First Semester (January - June) 2012 (Annex Business Electricity). Dublin, Ireland: Sustainable Energy Authority of Ireland; 2012.
- [18] Abbasi T, Tauseef SM, Abbasi SA. Anaerobic digestion for global warming control and energy generation—An overview. *Renewable and Sustainable Energy Reviews*. 2012;16(5):3228-42.
- [19] Astals S, Venegas C, Peces M, Jofre J, Lucena F, Mata-Alvarez J. Balancing hygienization and anaerobic digestion of raw sewage sludge. *Water Research*. 2012;46(19):6218-27.
- [20] Lloret E, Pastor L, Martínez-Medina A, Blaya J, Pascual JA. Evaluation of the removal of pathogens included in the Proposal for a European Directive on spreading of sludge on land during autothermal thermophilic aerobic digestion (ATAD). *Chemical Engineering Journal*. 2012;198–199(0):171-9.
- [21] Maya C, Torner-Morales FJ, Lucario ES, Hernández E, Jiménez B. Viability of six species of larval and non-larval helminth eggs for different conditions of temperature, pH and dryness. *Water Research*. 2012;46(15):4770-82.