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Review of the Integrated Process for the Production of Grass Biomethane

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Production of grass biomethane is an integrated process which involves numerous stages with numerous permutations. The grass grown can be of numerous species, and it can involve numerous cuts. The lignocellulosic content of grass increases with maturity of grass; the first cut offers more methane potential than the later cuts. Water-soluble carbohydrates (WSC) are higher (and as such methane potential is higher) for grass cut in the afternoon as opposed to that cut in the morning. The method of ensiling has a significant effect on the dry solids content of the grass silage. Pit or clamp silage in southern Germany and Austria has a solids content of about 40%; warm dry summers allow wilting of the grass before ensiling. In temperate oceanic climates like Ireland, pit silage has a solids content of about 21% while bale silage has a solids content of 32%. Biogas production is related to mass of volatile solids rather than mass of silage; typically one ton of volatile solid produces 300 m^3 of methane. The dry solids content of the silage has a significant impact on the biodigester configuration. Silage with a high solids content would lend itself to a two-stage process; a leach bed where volatile solids are converted to a leachate high in chemical oxygen demand (COD), followed by an upflow anaerobic sludge blanket where the COD can be converted efficiently to CH_4. Alternative configurations include wet continuous processes such as the ubiquitous continuously stirred tank reactor; this necessitates significant dilution of the feedstock to effect a solids content of 12%. Various pretreatment methods may be employed especially if the hydrolytic step is separated from the methanogenic step. Size reduction, thermal, and enzymatic methodologies are used. Good digester design is to seek to emulate the cow, thus rumen fluid offers great potential for hydrolysis.

1. Introduction

Digestion of grass silage (*1*, *2*) and resultant production of grass biomethane for use as a transport fuel in Ireland (*3*, *4*) has received substantial interest in recent years. The practice of producing and using grass biomethane as a transport fuel or as a displacement/replacement for natural gas in the gas grid is at an early stage in Germany and Austria (*4*, *5*). In Germany, codigestion of grass silage with maize silage (*6*) is common in agricultural biogas plants (*7*). The substantial increase in the use of grass as a cosubstrate is because of its high biodegradability and associated biogas production rates when compared to organic fraction of municipal solid waste (OFMSW) (*8*). In Ireland, farmers are considering the production of grass biomethane as an occupation in lieu of beef production, due to the low farm family income associated with animal husbandry (*4*). Furthermore, considering that 91% of Ireland's agricultural land is under grass, cross compliance rules (*9*) would decree that grass is the most ubiquitous bioenergy feedstock in an Irish context (*3*). Ireland's 9% arable land is fully utilized (*10*), which negates the potential for a bioenergy strategy based on energy crops. This paper reviews in detail the various processes and techniques involved in the production of grass silage for digestion, the potential for pretreatment, and digestion of grass silage.

1.1. Characteristics of Grass Silage. Grass silage can be utilized as a beneficial feedstock for the production of biomethane, especially in Ireland, due to its high yield (11–15 t dry solid (DS) ha^{-1} a^{-1}), its perennial nature (a low energy input crop), the high volatile solid (VS) content (ca. 92%), and the associated relatively high biomethane yield (*3*, *4*). The chemical composition of grass may be noted in Table 1. An ultimate analysis of grass silage was carried out by the authors (Table 2). The analysis yielded the following stoichiometric equation for dry grass: $C_{28.4}H_{44.5}O_{17.7}N$. This yields a carbon to nitrogen ratio (C:N) of 24:1. The energy content of grass silage based on the modified Dulong formula (*14*) is found (Box 1) to be 18.77 MJ kg^{-1} VS.

TABLE 1. Chemical Composition of Grass (*11*–*13*)

	grass
cellulose (%)	25–40
hemicellulose (%)	15–50
lignin (%)	10–30
ash (%)	1.5
C (%)	49.93
N (%)	2.05
K (%)	1.51
Ca (%)	1.91
Mg (%)	0.26
B (mg kg^{-1})	23
Cu (mg kg^{-1})	13
Fe (mg kg^{-1})	0.29
Zn (mg kg^{-1})	72
Na (mg kg^{-1})	915

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TABLE 2. Chemical Composition of Grass As Assessed by Authors

	atom wt	no. of atom	contribution	% contribution
C	12	28.4	340.8	49.93
H	1	44.5	44.5	6.52
O	16	17.7	283.2	41.49
N	14	1	14	2.05
total			682.5	100

Box 1. Energy content of grass silage

Formula for silage on dry matter basis = $C_{28.4}H_{44.5}O_{17.7}N$

From modified Dulong Formula (*14*);

Energy Content of fuel (kJ kg^{-1}) = 337C + 1419(H −1/8O) + 93S + 23.26 N

= 337(49.93) +1419(6.52−41.49/8) +23.26(2.05)

= 18,770 kJ kg^{-1}

Energy content of silage on dry and ash free basis (VS) = 18.77 MJ kg^{-1}

Cellulose, hemicellulose, and lignin are the three main types of polymers that constitute lignocellulosic materials like grass. The strong interlinkage of these polymers with noncovalent forces and covalent cross-linkage gives a stable shape and structure to the plant (*15*). Cellulose and hemicellulose are macromolecules consisting of the same or different carbohydrate units, while lignin is an aromatic polymer made by phenyl propanoid precursors (*16*). Secondary components are protein, lipids extracts, pectin, and nonstructural carbohydrates (*17*). The chemical composition of grass changes as the plant matures (*18*). For example, the lignin content starts increasing with plant maturity and growing season, i.e., after anthesis (Figure 1).

1.2. Anaerobic Digesters. The use of a two-stage digester configuration for high solid content feedstocks (such as grass silage) supports high growth rates of hydrolytic and methanogenic bacteria (*20*−*22*). In particular the incorporation of a high rate reactor, such as an upflow anaerobic sludge blanket (UASB) downstream of the initial digester, has been studied by workers including Lehtomäki and Björnsson (*23*) and Yu et al. (*24*). A viewpoint of Nizami and Murphy (*5*) is that sequencing batch leach-beds (SBLB) coupled with a UASB, and continuously stirred tank reactors again coupled with a UASB, offer great potential for grass biomethane production (Figure 2). These advantages include potentially higher organic loading rates (OLR) and methane yields at reduced digester volumes (*5*).

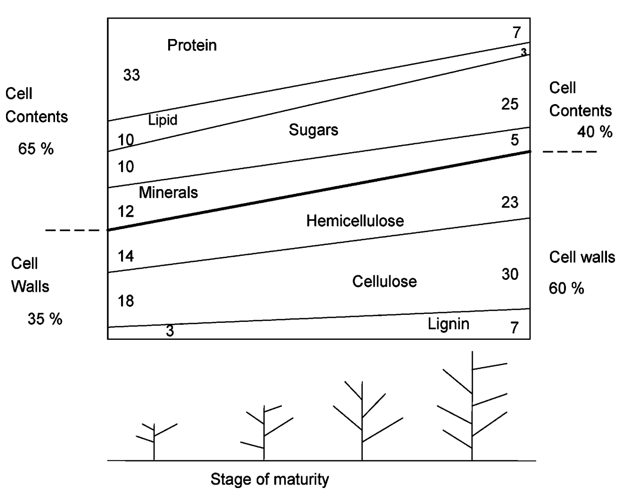

FIGURE 1. Chemical composition of grass with advancing maturity (*19*).

1.3. Anaerobic Biodegradation of Grass Silage. The crystalline structure of cellulose is a barrier in the penetration of microbes and enzymes into the cellulosic parts (*25*). Therefore, cellulose is resistant to hydrolysis while hemicellulose is weak to hydrolysis due to its amorphous structure. It is easily hydrolyzed by dilute acid or base as well as many hemicellulase enzymes (*26*). Lignin is the most recalcitrant part of the structural carbohydrates because of its nonwater-soluble nature; lignin is resistant to microbial action and oxidative forces (*27, 28*). Therefore, the biodegradability of grass silage in a digester is limited by the crystallinity of cellulose and lignin which is enhanced by the high contents of fiber (ca. 30%) in grass silage (*23*). Inefficient biodegradation results in reduced solubilization of grass silage, which limits the conversion of volatile solids to chemical oxygen demand (COD). In a system incorporating two stages with the first serving as a vessel for hydrolysis and acidogenesis and the second as a methanogenic stage, the production of COD in the first vessel is essential (*5*).

The protein, lipid, and extracted fractions of carbohydrates are the soluble parts of grass silage, while the fibrous components represent the structural carbohydrates of the plant, which are difficult to solubilize. Solubility of hemicellulose triggers cellulose accessibility (*28*) to enzymes and microbes. Considering lignin, another important constituent of structural carbohydrates, Lehtomäki et al. (*1*) stated that it is removed more efficiently due to solubilization than to degradation. This result is in line with the observation made by Kivaisi et al. (*29*). Thus, the biodegradation and solubilization of lignin and hemicellulose could lead to more efficient cellulose hydrolysis of grass silage.

In hydrolysis, cellulose is converted into the simple sugar glucose (*30*). Hydrolysis determines the range of loading and operational measures of the digester, as it holds a critical and rate limiting role in the anaerobic digestion of lignocellulosic substrate (*31*). A complex set of enzymes generally known as cellulases is associated with hydrolysis of grass silage, which further enables a range species of bacteria to work on cellulosic surfaces. Therefore, hydrolysis is considered first as an enzymatic hydrolysis and then microbial hydrolysis (*32*). The hydrolysis rates are determined by the attachment of hydrolytic enzymes to the surface of biodegradable material (*33*). These rates are accelerated by treating the substrates chemically, physically, and/or biologically prior to feeding to the digester (*34*). As a result, the structural and compositional barriers of grass silage to digestion such as the lignin seal, cellulose crystallinity, and degree of polymerization, are altered or removed. This enhances the efficiency of methane production (*35*).

1.3. Treatment; Pre/During/Post. Different treatment methods are used to overcome the limitation of hydrolysis of grass silage (*36*) based on biodegradation and solubilization of lignin and hemicellulose, with the aim of facilitating optimal methane production (*37*). The system of anaerobic digestion from the production of raw material to the end-use product is represented using a modification of circular diagrams (*38*) (Figure 3). In this methodical illustration the inter relationship between the components of inputs (agronomic factors, operational measures, and pretreatments) and of outputs (biomethane and digestate) from the digester are highlighted.

2. Anaerobic Reactors

2.1. Single-Phase Digestion. The anaerobic process including the configuration of the digesters is a crucial element of the process (*5*). Initially digesters tended to be simple, one-stage processes in which all stages of the anaerobic microbiological process were encouraged to coexist. Figure 2a indicates a single-phase digestion process; the process is divided into two chambers but both chambers have a complete array of anaerobic bacteria (hydrolytic, fermentative, obligate proton reducing acetogenic, acetoclastic, and hydrogenophilic methanogenic bacteria). This is based upon a facility visited by the authors in Eugendorf, Austria and documented by Smyth et al. (*4*). The facility digests silage at 42% DS. The silage is diluted to about 12% DS in the first chamber by return of leachate from the final chamber. A loading rate of 1.4 kg VS m^{-3} day^{-1} was utilized (*4*). Box 2 outlines the biogas production as 206 m^3 biogas t^{-1} silage. The methane production equates to 0.295 m^3 CH_4 kg^{-1} VS added. This value is as would be expected from the scientific literature (*3*–*5*). In an Irish context where pit silage is typically at 21% DS, the biogas production would be of the order of 100 m^3 t^{-1} (56 m^3 CH_4 t^{-1}) (*4*).

Box 2. Biogas production in single-phase digestion of grass (adapted from Smyth et al. (*4*))

One tonne of silage @ 42% dry solids = 420 kg VS t^{-1} @ 92% VS = 386 kg VS t^{-1}

60% destruction of volatiles = 232 kg VS_{dest} t^{-1} grass

1 kg VS = 18.77 MJ; 1 m^3 CH_4 = 37.78 MJ; Biogas @ 55.5% CH_4 = 21 MJ;

Thus destruction of 1 kg VS = 0.89 m^3 biogas @ 55% CH_4

Thus 60% destruction of volatiles in silage @ 42% dry solids yields:

206 m^3 biogas t^{-1} silage

114 m^3 CH_4 t^{-1} silage

CH_4 production per kg VS added yields:

0.295 m^3 CH_4 kg^{-1} VS added

Thus if silage from a pit at 21% dry solids is digested the expected yields of gas are as follows:

103 m^3 biogas t^{-1} silage

57 m^3 CH_4 t^{-1} silage

2.2. Two-Phase Digestion.

The two-stage process gained popularity, initially in the laboratory, as each bacteria stage enjoys different environmental conditions (*21*, *23*, *24*). Furthermore it was realized that certain stages may be limiting for certain feedstocks. In particular digestion of feedstock with high dry solids content and more particularly high lignocellulosic content is rate limited by hydrolysis (*39*).

Figure 2b highlights a two-phase system whereby the acidogenesis stages (hydrolytic and fermentative) are separated from the methanogenic stages. Figure 2c highlights a system that incorporates leaching of high solids content feedstock (such as grass silage) to a leachate/wastewater high in COD. The leachate is treated in an upflow anaerobic sludge blanket (UASB) which may be subjected to very high organic loading (up to 20 kg COD m^{-3} d^{-1}) while effecting a 90% destruction in COD (*40*). The digested leachate is returned to the leach beds to generate more COD. This system may be termed a sequencing batch leach bed reactor complete with UASB (SBLB-UASB). In Box 3 the potential for COD production is estimated and the associated CH_4 production is calculated. The same silage (42% DS and 92% VS) (*4*) is utilized in the calculation. It is shown in Box 2 and Box 3 that if 67% of volatiles are destroyed by the digestion process the same gas production (114 m^3 CH_4 t^{-1} silage) may be achieved as by the single stage process. This is to be expected as 60% of volatiles are destroyed in the single-phase system; in the SBLB-UASB 90% of COD is converted, thus 67% of volatiles must be destroyed (60/0.9). The potential for this system is the higher loading rate achievable in the UASB (20 kg COD m^{-3} day^{-1}) versus the single-phase system (1.4 kg VS m^{-3} day^{-1}) (*4*). Allowing for 1.4 kg COD kg^{-1} VS^{-1} the UASB may undergo OLR 10 times higher than the single-phase process. Thus for optimal methane production within a reactor, the critical step is the hydrolysis and fermentation of the grass silage in the leach beds. This is dependent on the grass production process, the operation and environmental conditions within the digester, and pretreatments employed as discussed in the following sections.

Box 3. Biogas production in two phase digestion

Relationship between VS and COD

Based on energy basis

Energy content of methane = 37.78 MJ m^{-3}

Energy content of silage = 18.77 MJ kg^{-1} VS

From Sperling et al. (*41*), 1 kg COD produces = 0.35 m^3 CH_4

Energy content = 0.35 × 37.78 = 13.22 MJ kg^{-1} COD

Relationship of VS and COD = 18.77 MJ kg^{-1} VS/13.22 MJ kg^{-1} COD

= 1.42 kg COD kg^{-1} VS

Based on chemical reaction

The basis for the COD test is that nearly all organic compounds can be fully oxidized to carbon dioxide with a strong oxidizing agent under acidic conditions. The amount of oxygen required to oxidize an organic compound to carbon dioxide, ammonia, and water is given by:

$$C_nH_aO_bN_c + ((n + a)/(4 - b)/(2 - 3c)/4)\ O_2 \rightarrow nCO_2 + (a/(2 - 3c)/2)\ H_2O + cNH_3$$

For silage @ 42% DS, 92% VS:

$$C_{28.4}H_{44.5}O_{17.7}N + 29.925\ O_2 \rightarrow 28.4\ CO_2 + 20.75\ H_2O + NH_3$$

For 1 kg silage: 0.3864 kg VS + 0.542 kg O_2 →...

Therefore, the relationship is 1.40 kg COD kg^{-1} VS

Biogas potential @ 42% DS and 92% VS

1 t silage @ 42% DS, 92% VDS = 386.4 kgVS

For 67% VS_{dest}: 386.4 × 0.67 = 258 kg VS_{dest} = 362 kg COD

For 90% removal of COD: 362 × 0.9 = 325 kg $COD_{removed}$

As 1 kg COD produces 0.35 m^3 CH_4;

1 t silage produces = 325 × 0.35 = 114 m^3 CH_4

→ 0.295 m^3 CH_4 kg^{-1} VS_{added} → 0.44 m^3 CH_4 kg^{-1} VS_{dest}

3. Agronomic factors

3.1. Potential Grass Types.

Depending on usage of grass either as a feed for livestock or as a feedstock for an anaerobic digestion process, the agricultural production of grass differs. This difference is due to the diversity of environmental and operational measures in growing and harvesting grass, and the microbiology of the anaerobic digester as opposed to rumen (*42*). For example, the level of cellulose degradation is up to 80% in biogas plants (*43*) with retention times of 30–80 days, while according to Gray (*44*) it is 40–60% in rumen with retention of about 2 days. To make grass silage more suitable for a digester, a different harvesting frequency and time may be required.

In the temperate grassland region, particularly in Ireland, the perennial ryegrass (*Lolium perenne*) is preferred for anaerobic digestion because of high digestibility values (*D*-values) (*45*), water-soluble carbohydrates (WSC) levels (*46*), and reduced quantities of crude fiber (Table 3). *D*-Value may be equated to the potential digestibility of the silage in cattle paunch (*45*, *47*). In work by Mähnert et al. (*2*) perennial ryegrass gave the highest biogas yield (0.83–0.86 m^3 kg^{-1} VS added), compared to other grass species both fresh and ensiled. For example *Dactylis glomerata* (cocksfoot) gave a biogas yield of 0.65–0.72 m^3 kg^{-1} VS added. Other grasses used as a substrate for a digestion process include *Phleum*

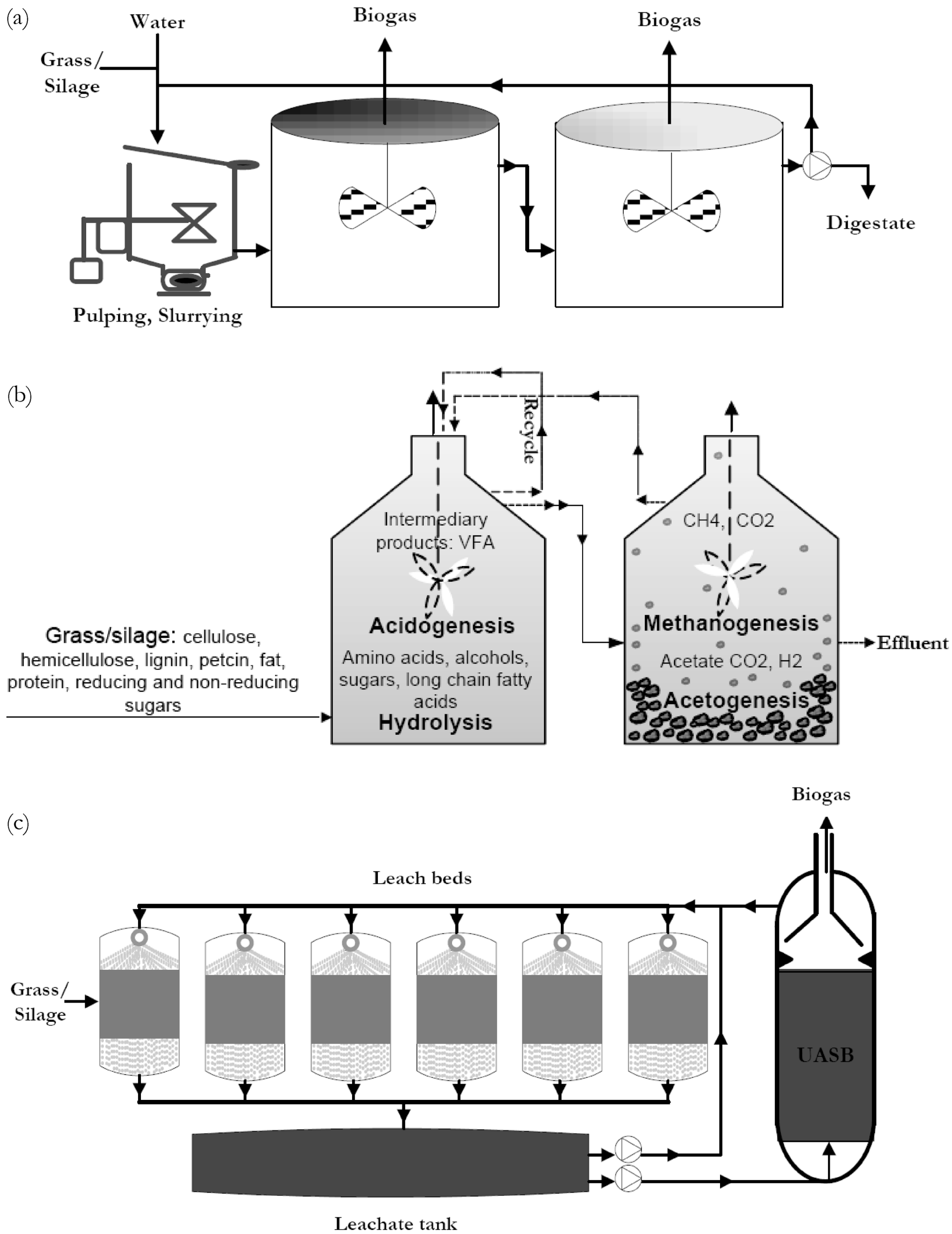

FIGURE 2. (a) Two-stage continuously stirred tank reactor; (b) two stage two phase digester; (c) sequencing batch leach bed reactors complete with UASB (SBLB-UASB) (*5*).

pratense (Timothy) and *Lolium multiflorum* (Italian ryegrass). Tetraploid ryegrass varieties are recommended due to high sugars levels (*47*). In recent times diploid varieties have tended to dominate mixtures in Ireland, but tetraploid varieties remain an important component of grass seed mixtures because of their higher WSC content, their increased palatability that determines higher intake by livestock, and their tolerance to drought. However, they tend to have lower tiller densities resulting in more open swards and lower dry matter compared with diploids. Seeding rates for tetraploid grasses will need to be higher because of their larger seed size (*48*).

Mixtures of grasses and grass silage increase methane yield as when compared to a single grass type such as in *Cynodon* spp. (Bermuda grass) (*20*). Additionally, Plochl and Heiermann (*49*) reported methane production from forage and paddock mixtures of 297−370 and 246 $m^3\ t^{-1}$ organic dry matter (ODM), respectively. Prochnow et al. (*50*) reported methane yields of 370 L kg^{-1} VS for ensiled mixture of grass and clover, cut before anthesis (mid-May) compared to after anthesis stage (mid-June). According to the same authors, feedstock from late cut yielded less methane when compared to the first cut because of the increased crude fiber content in comparison with the first cut. The efficiency of anaerobic digestion can be considerably improved in mixed feedstock such as that of grass with legumes because the neutral detergent fiber (NDF) concentration of grasses is usually greater than that of legumes, which is caused mostly by differences in NDF concentration of grass and legumes leaves (*51*). Hence, increasing the proportion of legumes and consequently the leaf to stem ratio of forage results in lower cell wall concentration, in other words reduced indigestible material and increases in digestibility of feedstock. Additionally, the benefits of grass−clover swards mixtures as stated by Stinner et al. (*52*) can be extended when the digestate from the anaerobic digestion process is relocated back to the field resulting in higher nitrogen (N) availability to the plants due to the transformation of digestate organic N to readily available ammonia-N.

3.2. Harvest Date and Frequency. Grass for silage is usually harvested at a less mature stage of growth (leafy and nonlignified) (Table 4) since the aim is to obtain a crop with a relatively high content of fermentable substrate and a low

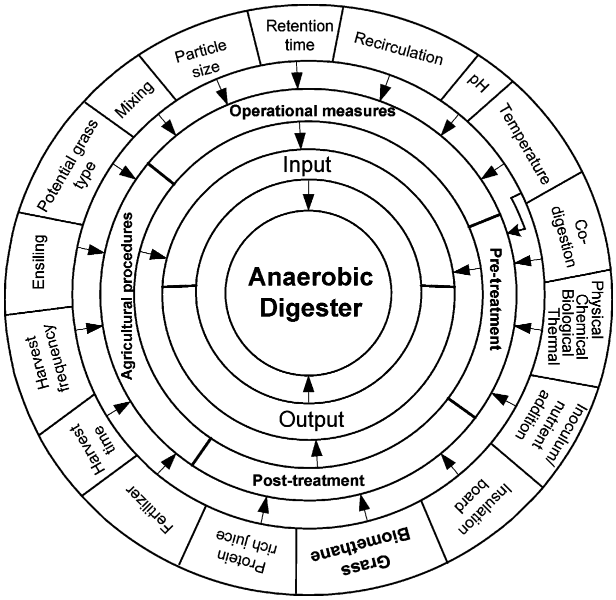

FIGURE 3. Circular diagram of the main factors determining grass biomethane yield of an anaerobic digester.

content of fiber. The crop at this stage usually has a high leaf−stem ratio (*54*); similar findings were made by Gunaseelan and Nallathambi (*20*) using leaves of *Pennisetum purpureum* (Napier grass). In a study by Amon et al. (*42*) on a multifaceted crop rotation to increase the yield of methane per hectare, the first cut at vegetation stage is selected as an optimum option for harvesting. Furthermore, De Boever et al. (*55*) found significant increases in structural carbohydrates (i.e., NDF and acid detergent fiber (ADF)) and lignin between early and late first cut in a permanent pasture consisting of 50:50 ratio between diploid and tetraploid varieties of perennial ryegrass. The same was observed in timothy regarding NDF at two different locations within three years of experimentation (*56*).

Production of methane per VS depends on harvesting time and species (*Trifolium* spp., *Lolium perenne*, *Phleum pratense* and *Trifolium* spp. in mixed swards) since the results produced by various experiments were not consistent. As such Kaparaju et al. (*57*) found that *Trifolium* spp. (clover) produced 50% more methane per VS at vegetative stage than at flowering stage. The results obtained were different when the same experiments were performed by Pouech et al. (*58*), where 32% lower methane yield per VS was recorded at vegetative stage than at flowering stage. Dieterich (*47*) imposes significant differences on the digestibility of grass silage which was reduced between first cut and second cut. Additionally, in Orkney (island north of Scotland) the *D*-value and metabolizable energy (ME) value for early cut silage (early June) were 67% and 10.7 MJ kg^{-1}, while the values for late cut silage (late June) were 65% and 10.4 MJ kg^{-1}, respectively (*47*). Prochnow et al. (*50*) described more biogas yield in second cut silage than first cut. But, in spite of high DS and VS contents present in late cut grass, a lower methane yield was established (*50, 59*). The total solid (TS) and VS content of grass depend on several factors such as location and origin, seasonal variations, cultivation practices, type of soil, pretreatment of the biomass, and nutrient composition of the grass (*60*); all of which affect the production and yield of grass biomethane (*5*). The methane content can also be increased if grass is cut in the afternoon as it increases the concentration of WSC. It has been stated by White (*61*) that the concentration of total nonstructural carbohydrates in several grass species when investigated was lowest at 6 a.m. and increased linearly to a high at 6 p.m.

Another important factor affecting the qualitative characteristics of grass silage related to harvesting management is harvest frequency. Forage quality among others is a function of high nutrient value and digestibility and is usually determined by animal performance when forages are fed to livestock (*62, 51*). Even more ideal pasture management would maximize both the quantity and quality of forage available to livestock (*63*). The authors cannot find any reason why the same indices cannot be used for silage evaluation as a feedstock for biomethane production particularly when experimental data regarding this important husbandry factor in relation to biomethane production are lacking. Geber (*64*) showed a decrease in dry matter yields and the dry organic matter with more than two harvests per year in case of *Phalaris arundinacea* (reed canary grass). According to Holliday (*65*), the 2 or 4 week cycle for cutting of grass is considered optimum for anaerobic digestion in terms of their C:N ratios; this cycle will also lead to a reduction in float or scum formation. However, Murdoch (*66*) has reported that an early first cut followed by cutting intervals of about six weeks will produce grass silage of high digestibility, whereas Motazedian and Sharrow (*63*) reported reductions in digestibility and crude protein in mixed pastures comprising of ryegrass and *Trifolium subterraneum* (subclover) as the defoliation interval increased from 7 to 49 days. Finally, intensity of harvest in case of mixed pastures (*67*), old or hill pastures (*63*), or even abandoned land can affect botanical composition, a perspective that merits further investigation since it could be used for efficient utilization of this land type considering the energy potential of the existed species for biomethane production along with biodiversity issues. In support of this argument Keating and O'Kiely (*68*) reported lower WSC in old pastures in comparison with pastures dominated with *Lolium multiflorum* and *L. perenne*.

3.3. Ensiling of Grass. Grass and in particular grass silage form the basal diet for the vast majority of ruminants in many parts of the world during the winter feeding period (*69, 70*). Lee et al. (*71*) stated that good quality silages will support high levels of performance in animals without the need of additional concentrate supplementation. Taking under consideration the potential of grass and grass silage as a feedstock to biomethane production as a biofuel (*3*) and the need to increase biofuel penetration in line with the European Directive for the use of biofuels (EC/30/2003), then this necessitates the rapprochement of grass and grass silage production and their characteristics that make them suitable for both feed and biofuel.

To keep a constant quality and supply of substrate to an anaerobic digester facility, ensiling of grass as silage is preferable to utilization of fresh grass. In addition, grass silage produced higher methane per tonne of ODM than fresh grass (*65*). There may be potential to batch digest fresh grass in the summer months and to utilize other energy crops or biomass in the winter months. Ensiled grass in comparison to dried and stored grass ensures lower organic matter losses and independency of weather conditions that might cause damages to the dried feedstock (*72*). During ensiling the resistive polysaccharides are degraded and intermediates such as volatile fatty acid (VFA) for methanogens are produced, which increase methane yield in the digester (*73*). Mshandete et al. (*74*) reported that initial high concentrations of VFA (especially propionic acid) negatively influences the microbial population of methanogens, while at the same time VFA can also be toxic to the anaerobic process. Based on evidence from animal production research, VFA can account for up to two-thirds of the ruminant energy requirement (*55*), although according to Bannink (*75*) a significant proportion of VFA is absorbed by cells of epithelial tissues in the rumen wall and is not used as energy source, resulting in less efficient use of the feedstock by cattle.

TABLE 3. Comparison of Fresh and Ensiled Grass Characteristics in Batch and CSTR Digesters (*2*)

	batch digester					CSTR			
	fresh grasses			grass silage		fresh grasses			
	perennial ryegrass	cocksfoot	meadow foxtail	perennial ryegrass	cocksfoot	perennial ryegrass	cocksfoot	meadow foxtail	mixture
TS (% FM)	17.6	18.6	15.8	18.7	27.3	25.6	22.9	24.2	24.2
VDS (% TS)	90.1	89.1	91.1	88.5	88.8	90.6	88.8	90.6	90.0
VFA (g kg^{-1} FM)	0.5	0.5	0.3	6.9	14.3	0.7	0.5	0.6	0.6
pH	6.5	6.7	6.6	4.6	6.1	6.5	7.1	7.1	6.9
C:N	16.4	13.7		15.5	14.3	19.8	12.0	13.5	15.1
XP (% TS)	14.7	18.5		17.0	18.4	11.8	21.4	18.8	17.4
XF (% TS)	24.8	24.8	25.3	31.3	30.1	29.1	28.0	31.5	29.5
Saccharides (% TS)	10.8	9.8	3.3	3.4	3.1	19.3	9.8	9.1	12.7
XL (% TS)	2.1	2.3	2.2	4.9	4.6	2.4	2.6	2.1	2.4

However, this is specific to the rumen and may not be significant to biomethane production; hence several issues still need to be addressed:

- the mechanism of microbial fermentation, with conversion of feed matter into large amounts of VFA is critical for a correct description of such systems;
- conservation of WSC as a higher resource of energy as compared to VFA (*70*) allowing increased potential for higher biomethane production;
- ensiling that results in less VFA production through methods such as wilting (*51, 69, 76*) or the use of additives such as formic acid (*76*) merits more attention.

Nevertheless, the use of additives in silage preparation did not increase methane as recorded by Neureiter et al. (*77*), Madhukara et al. (*78*), and Rani and Nand (*79*). Conversely, according to Lehtomäki (*25*) formic acid addition resulted in higher methane production.

During ensiling, nutrient losses occur due to plant respiration, fermentation, and storage. Aerobic respiration involves the action of enzymes on the carbohydrate fraction of the herbage and consequently produces water, carbon dioxide, and heat. If sufficient oxygen is present two major effects can occur. The first is the reduction in carbohydrate supply, which restricts the quantity of lactic acid formed in the silage. Second, the heat produced raises the temperature of the silage and, if this rises above 40 °C, the digestibility of crude protein can be reduced markedly (*66*). Moreover, there is a need to restrict clostridia growth that consumes lactic acid (*66*) and causes deterioration in silage quality (*54*). Ammonia may act as a simple index of silage fermentation quality (*70*) since it is predominantly a product of clostridia fermentation of amino acids and its excess indicates a low quality product. Keady et al. (*80*) reported that improvements in silage fermentation could be indicated by decreases in pH and ammonia-N due to various factors such as use of additives, e.g., formic acid. Additionally, the formation of monosaccharides starts the hydrolysis during ensiling. Therefore, acidic conditions are suggested during the whole process of ensiling (*81*) to produce efficient silage for the digester. Conservation of grass can be in the form of silage in bales, pit, and/or clamp and hay. In an Irish context bale silage tends to have a solids content of 32% while pit silage has a solids content of 21%. A bale of silage has a mass

TABLE 4. Analysis of Grass Silage Characteristics (*1, 23, 53*)

parameter	measuring unit	range
dry matter digestibility (DMD)	g kg^{-1}	780 (excellent leafy stage) 500 (poor late cut silage)
acid detergent fiber (ADF)	g kg^{-1} DM	220−450
neutral detergent fiber (NDF)	g kg^{-1} DM	400−750
ash	g kg^{-1} DM	<100
crude protein	g kg^{-1} DM	>160 (leafy young tissue) 100−160 (grass for silage) <100 (very stemmy herbage)
pH	–	3.8−4.2 (wet silage) 5.0 (wilted silage) 6.0−6.3 (fresh herbage)
lactic acid	g kg^{-1} DM	80−120 (well preserved) <50 (poorly preserved)
volatile fatty acids (VFA)	g kg^{-1} DM	acetic acid: 20−50 (well preserved) butyric acid: < 10 (good quality) propionic acid: < 10
ammonia-N (g/kg of total N)	g kg^{-1} N	<100 (well fermented) 150−200 (poorly fermented)
carbohydrates	(% TS)	45.0
extractives	(% TS)	8.4
proteins	(% TS)	10.4
SCOD	(mg g^{-1} TS)	228
N_{tot}	(mg g^{-1} TS)	16.9
crude fiber	(% TS)	20.5
NDF	(% TS)	28.7
ADF	(% TS)	28.8
lignin	(% TS)	5.4

of 660 kg and contains the same dry matter as one tonne of pit silage (211 kg DS) (*4*).

4. Operational Procedures

4.1. Temperature. Generally, increases in temperature result in higher levels of solubilization (*13*) since xylan, the major component of hemicellulose in grass, is not stable at high temperatures (*28*). Therefore, short-chain fragments are formed due to increased temperature, resulting in higher biological suitability of substrate to microorganisms (*8*). The literature contains contradictory evidence and variations on the benefits of mesophilic or thermophilic temperature ranges. One explanation of these variations is when temperature shifts from mesophilic to thermophilic, a time period is required to ensure that a sufficient microbial population has grown, otherwise it will result in a decrease in production of biogas and methane (*34*). In a study by Bouallagui et al. (*82*) more than 95% VS is removed to produce methane at rate of 420 L kg^{-1} VS added, when the first and second stage of the digester were operated at thermophilic and mesophilic temperatures, respectively. The problem in using thermophilic temperature ranges in the digester is the high parasitic energy demand (*34*). Therefore, if two-stage and two-phase digestion is used (Figure 2b and c) the first stage should be operated at thermophilic temperatures and the second stage should be operated at mesophilic temperatures to accelerate the grass hydrolysis and ultimately the methane yield.

4.2. pH Range. According to Ward et al. (*34*), the most suitable pH for anaerobic digestion is 6.8−7.2. Below a pH level of 6.6, the methanogenic population decreases. Excessive alkalinity also results in the failure of the process by disintegration of microbial granules (*83*). pH values for the first and second stage of two-stage two-phase digesters vary according to Yu et al. (*24*) and Kim et al. (*84*). According to Yu et al. (*24*), in the first stage of a grass digester the pH should be between 4.0 and 6.5. However, Dinamarca et al. (*85*) and Babel et al. (*86*), both working on solid waste, both stated that a pH less than 7 in the hydrolysis tanks did not enhance the hydrolysis rate. In the second stage of a two-phase digester the pH should be around 7.0 (*84*). Increases of pH in the leachate tank of the SBLB (Figure 2c) to 7.2 indicate the beginning of methanogenesis and methane production (*87*). The pH alters when the total VFA concentration exceeds 4 g L^{-1} and glucose is inhibited for fermentation (*88*). A constant pH in two-stage digestion can also be maintained by modifying the inoculum to feed ratio (*89*).

4.3. Mixing. Gentle mixing increases digester stability against shock loading; it also increases availability of substrate to bacteria and improves methane yield (*90*). Excessive mixing reduces the oxidation of fatty acids due to disruption of granulation (*91*), which is formed from protein and carbohydrate extracellular polymers (*92*). If the substrate is mixed with support media such as carbon filter, rock wool, loofah sponge (*93, 93*), pored glass material (*94*), or clay minerals such as sepiolite and stevensite (*95*) higher levels of SCOD are achieved (*96*). Support media are often used in high-rate digesters for wastewater/leachate treatment, such as anaerobic filters; these are often used as the second stage of a two-phase process in lieu of a UASB (Figure 2c). Mixing the digestate with fresh substrate improves bacterial performance (*97*). There is a need to use a paddle mixer in continuously stirred tank reactors (CSTR) to overcome floating or scum formation when the dry matter of the feedstock is below 5−6% (*47*). Gas mixing is one efficient option especially for reactors treating plant biomass. Gas mixing is also used as a mixing option in dry continuous digesters such as “Volarga” systems (*5*).

4.4. Particle Size. Particle size can affect methane yield significantly because of the increased availability of surface area for fiber degradation through hydrolyzing enzymes and bacteria (*34*). According to Mshandete et al. (*98*), methane yield increased when the size of the particles reduced from 100 to 2 mm whereas the threshold limit of particle size, particularly for grasses, was set at 0.40 mm by Sharma et al. (*99*), at 1 mm by Chynoweth et al. (*100*), at 3 mm by Braun (*101*) and at 10 mm by Kaparaju et al. (*57*). In case of *Cynodon dactylon* (Bermuda grass) the least effect was found at a size of 0.40 mm; below there was no effect on biogas production until a size of 0.088 mm was reached. Parasitic energy demand dictates against reduction in size of grass silage below 1 mm in commercial-scale plants (*20*).

4.5. Retention Time. In a study of agricultural solid residues by Demirbas and Ozturk (*97*), 80−85% of biogas was produced in the first 18 days of a total of 30 days digestion period. Qi et al. (*13*) also proposed a period of 2−3 weeks as an optimum HRT for lignocellulosic substrates. Silvey et al. (*102*) suggested that the batch leach bed digester should be loaded for 18−30 days, rather than 60−90 days when digesting unsorted municipal solid waste. Demirbas and Ozturk (*97*), state that the composition of VFA in shorter or longer digestion periods does not change significantly. This agreement on an approximate 30-day HRT is also supported by Lehtomäki and Björnsson (*23*), when they obtained 85% of total methane production from grass within 30 days, using a two-stage batch digester at pilot scale. However, Chugh et al. (*103*) obtained 95% total methane within 45 days digesting unsorted solid waste. This is further testified in a study by Lehtomäki et al. (*1*), using a leach bed digester with UASB, operating at 55 days retention time using grass as a feedstock. They recorded 66% of methane potential, while 39% of carbohydrates were removed after 49 days retention time. The relationship between OLR and HRT is important. Silage may be at 40% dry solids content in Austria or Germany when wilted or 22% dry solids content in Ireland when pit silage is produced. The retention time must be decided by the mass of volatiles added rather than the mass of silage added. An operating facility visited in Austria used a loading rate of 1.4 kg VS m^{-3} reactor day^{-1} (*4*).

4.6. Codigestion. Methane production is improved by codigesting grass silage with manure (*104*) even though the C:N ratio of grass makes it a suitable substrate as a monosubstrate (*5*). Hashimoto (*105*) found increased microbiological stability and buffering capacity in combination with reduced nutrient deficiency when converting straw to biomethane with manure as a cosubstrate. Macias et al. (*106*) outlined the positive role of cellulase enzymes and methane bacteria present in manure on the digestion process. Additional nitrogen can be supplied by codigestion with manure (*107*) or urea or food wastes (*97*). Codigestion of grass silage with slurries improves the digestion process through a reduction of ammonia and H_2S in the digester; both of which are inhibitors to the digestion process in elevated quantities (*108*). In a study by Lehtomäki et al. (*109*), 53% VS removal was achieved at methane yield of 0.268 m^3 CH_4 kg^{-1} VS added in codigestion of grass with cow manure in the ratio 2:5. Enhanced hydrolysis rates were observed by Qi et al. (*13*) in a study codigesting turf grass with activated sludge in batch and 2-stage semicontinuous digester configuration at laboratory scale.

4.7. Nutrients, Inoculum, and Inhibition. Gunaseelan and Nallathambi (*20*) found a 40% higher methane production and reduced VFA concentration with the addition of nickel (Ni), cobalt (Co), molybdenum (Mo), selenium (Se), and sulfate. Nutrients may be added if the methanogens need some supplementary trace elements such as Ni, Co, Mo, and Se (*110*). The ideal nutrient ratio for hydrolysis and acidogenesis is C:N:P:S of 500:15:5:3 and for methanogenesis is 600:15:5:3 (*47*). Different additives can also increase the production rate of methane. However, they need to be

TABLE 5. Effects of Various Pre-Treatments on Lignocellulosic Substrates[a] (*28, 35*)

	accessibility of surface areas	cellulose crystallinity	hemicellulose solubilization	lignin solubilization	lignin structure alteration	formation of furfural/HMF
mechanical	+	+				
steam pretreatment/steam explosion	+		+	−	+	+
LHW (batch)	+		+	−	−	−
LHW (flow through)	+		+	+ −	−	−
acid	+		+	−	+	+
alkaline	+		−	+ −	+	−
oxidative	+			+ −	+	−
thermal +acid	+		+	+ −	+	+
thermal +alkaline (lime)	+		−	+−	+	−
thermal+ oxidative	+		−	+ −	+	−
thermal +oxidative +alkaline	+		−	+ −	+	−
ammonia (AFEX)	+	+	−	+	+	−
CO_2 explosion	+		+			

[a] (+) Major effect. (−) Minor effect. (Blank) Not determined/yet unknown.

evaluated based on financial terms (*34*). By supplementing grass silage where nutrient deficiency is evident, an increase in methane production may be observed. For example, digesting Bermuda grass increased methane production by 96% with the addition of NH_4Cl (*20*).

In the presence of large quantities of inoculum in the second stage of a two-phase process such as a SBLB-UASB system (Figure 2c), the process is very stable, high methane yields are achieved, and pH adjustment is not required (*20*). This statement is in line with the observation made by Lehtomäki et al. (*1*) digesting grass silage in a two-phase process.

Ammonia, sulphide, light metal ions, and heavy metals are some of the different inhibitory substances for anaerobic digesters (*111*). During digestion of energy crops, the nitrogen discharged from plant protein in the form of free ammonia and ammonium is an inhibitory substance (*59*). When short-chain fatty acids are in higher concentration, they inhibit methanogens. An increase in VFA is an indicator of overloading rate (*34*).

4.8. Recirculation of Leachate/Water. Hydrolysis of organic matter was found to be accelerated by the recycling of leachate from the methanogenic process in an UASB to the first reactor in a two-stage/two-phase system (*112*). Initially, an increase in VFA and COD levels accompanied by a reduction in pH levels was observed. According to Sponza and Ağdağ (*113*), and Lai et al. (*114*) the optimum period of leachate recirculation for efficient digestion is a recirculation frequency of 4 times per week; when the leachate volume is equal to or greater than 10% of the bed volume the frequency is 7−12 days. As Demirbas and Ozturk (*97*) stated the supplemental nitrogen is converted to water-soluble ammonium after its release from the digested matter, thus by recycling of leachate the need for more nitrogen is also reduced.

5. Pretreatments Options

5.1. Pretreatment. According to Qi et al. (*13*) a combination of different pretreatment approaches with different operational procedures is required for optimum cost-effective pretreatment manipulation accompanied with minimum parasitic energy demand. Besides the pretreatment application attention should be focused to avoid any excessive formation of inhibitory byproducts such as furfural, hydroxymethyl furfural, and levulinic acid (*115*). For lignocellulosic substrates, economically viable and operationally efficient pretreatment options include steam pretreatment, lime pretreatment, liquid hot water (LHW), and ammonia-based pretreatments (Table 5). Less optimal options include concentrated acid, wet oxidation, solvents, and metal complexes (*35*). The established methane potential of grass silage without pretreatment in many studies was recorded at 0.3 $m^3\ kg^{-1}$ VS added (*20*). This is in line with the results in Box 2 and Box 3.

5.2. Physical Pretreatment. Physical/mechanical pretreatments increase pore-size of grass silage by releasing intercellular components (*116*). Particle size and available surface area in combination with pore size of the substrate in comparison to the size of the enzymes is also a limiting factor in hydrolysis (*117*). With increasing pore size, the hydrolysis of hemicellulose increases, which further accelerates cellulose hydrolysis (*118*) and lignin degradation (*119*). Drying is not favorable after pretreatment because this causes the collapse of pore structure, which reduces hydrolyzable substrates (*120*) in the digester. The milling effect can increase methane content from 5 to 25% (*28*) but higher parasitic demands make milling treatment less attractive (*121*).

5.3. Chemical Pretreatment. Chemical pretreatment increases surface accessibility for enzymes and bacteria by decreasing cellulose crystallinity (*122*). In chemical pretreatment, application of NaOH, NH_4OH, or a combination of both to grass fiber increases the potential of methane yield (*8*). Acid pretreatment might be a preferential choice for grass silage because of the enhanced degradation of xylan (the major component of hemicellulose) in acidic environments (*28*). Formic acid can be used as ensiling method, but it also serves as chemical pretreatment. The reactor configuration is of essence in this pretreatment option. Acid pretreatment is a preferred choice only in the first stage/phase of a two-phase process such as the leach bed in a SBLB-UASB (Figure 2c), due to methanogenesis in the UASB unit which can regulate any possible incoming inhibitory compounds, i.e., furfural and HMF (*123*). However, lower methane yield is observed when sulphuric or nitric acids are used due to formation of H_2S and N_2 (*28*). An increase of 100% in the yield of methane was observed due to alkaline pretreatment when wheat straw was used as a digester substrate (*39*). Although, according to Pettersen (*124*), this could lead to the formation of denser and more thermodynamically firm cellulose than innate cellulose. Therefore, the use of alkaline pretreatment in a CSTR digester can cause formation of toxic compounds which degrade acetate and glucose 15% and 50%, respectively, through the saponification reaction (*125*).

5.4. Thermal Pretreatment. Thermal pretreatment affects the degradation of lignin (*126*) and hemicellulose which further increases hemicellulose hydrolysis by the acids formed from the thermal treatment (*28*). Inhibitory or toxic effects may be caused to bacteria, yeast, and methanogens due to the phenolic compounds produced from the solubilization of hemicellulose and lignin at 160 °C (*127*). Recondensation and precipitation on feedstock may result if soluble lignin compounds are not removed quickly (*128*). The composition of the lignocellulosic substrates determines the thermal reactivity (*129*). Thermal pretreatment may be divided into two categories: liquid hot water and steam pretreatment.

5.4.1. Liquid Hot Water (LHW). LHW solubilizes hemicellulose, thus enhancing the accessibility of cellulose (*28*). One advantage of using LHW in a two-phase process (such as a SBLB-UASB digester) is to achieve higher concentrations of soluble carbohydrates such as xylan (*128*); degradation of xylan is more effective by the use of LHW as compared to steam pretreatment (*130*). The increase in enzymatic hydrolysis of the lignocellulosic material is increased 6-fold after LHW treatment. Due to higher water input in LHW, the concentration of soluble hemicellulose and lignin are reduced (due to dilution) in comparison to steam pretreatment, hence the risk of condensation and precipitation of lignin is reduced (*28*).

5.4.2. Steam Pretreatment. Steam pretreatment (*120*) loosens cellulose fiber by removing large fractions of hemicellulose. This results in increased accessibility of the enzymes to cellulose (*131*), although as Hendriks and Zeeman (*28*) mentioned this method could yield lower methane volume due to condensation and precipitation of lignin over lignocellulosic substrate.

5.5. Biological Pretreatment. Biological pretreatment offers a cost-effective solution in comparison to other pretreatments but it requires a specific environment to work efficiently (*34*). As an example, treatment with cellulase enzymes (*26*) during silage preparation caused an increased degradation of plant cell wall constituents that were more susceptible to bacterial decomposition. Additionally, Ridla and Vehida, Sajko et al. (cited in Clavero and Razz, (*26*)) reported that the addition of cellulase enzymes facilitated the breakdown of a component of structural carbohydrates during ensiling which resulted in improved degradation during silage fermentation. Considering the use of inoculants it has been proposed that heterofermentative bacteria (as compared to homofermentative bacteria) could be more beneficial for efficient anaerobic digestion, since they facilitate the production of intermediates for methanogens (*54, 132*). Additional benefits include the reduction in quantity of digestate and reduced parasitic energy demands (*133*). The use of the filamentous fungi, especially white-rot fungi, as a biological pretreatment has been studied recently due to its potential to degrade lignin (*16*).

5.6. Combining Pretreatments. Combined pretreatment at thermophilic temperature ranges (55 °C) (*13*) in the first phase of a two-stage/phase system could prove an efficient solution in hydrolysis of grass silage feedstock. Physiochemical pretreatments can increase solubility of substrates (*8*). Size reduction and preincubation with hot water have a positive effect in accelerating hydrolysis (*20*).

6. Research Required to Improve Grass Digestion

There is a wealth of research waiting to be undertaken in the area of agronomy, particularly in optimization of ensiling, grass types/mixtures, and harvest time. The list below is a few areas we feel are relatively important but not often discussed in the literature of anaerobic digestion.

6.1. Process Control System. Commercial scale anaerobic digesters are often run outside their optimum loading rates due to less effective monitoring and control mechanisms (*34*). This can trigger process instability in digesters by forming inhibitory substances, such as elevated fatty acid levels which effect methanogens (*134*). Therefore, online closed-loop monitoring through sensory devices, data-reading software, and automated control are beneficial. Research in the application of nanotechnology in monitoring and controlling process parameters (such as pH, temperature, VFA, COD, etc.) through chips and sensors at laboratory/pilot-scale digesters will allow for new ways to overcome inhibitory effects in digesters operating at industrial/commercial scale.

6.2. Rumen Fluid/Saliva. It should be borne in mind that cattle can breakdown the volatile solids in grass silage to a level of over 50% in 2 days. This may be compared with 60% destruction of volatile solids with a retention time of over 60 days in a CSTR digester in Austria (*4*). Rumen fluid increases the formation of fatty acids (*135*) which stimulates grass silage biodegradation in leach bed/hydrolyzing tanks. Rumen microorganisms are a promising source of inoculum in methanogenic stages of two-phase processes. Rumen microbes convert carbohydrates into energy-producing substances such as acetic, propionic, and butyric acids (*136*). Therefore, the use of rumen fluid/saliva in accelerating hydrolysis rates in leach beds and CSTR, and cow dung as inoculum in the second phase of two-phase digestion, has a significant potential to enhance methane yield.

6.3. Use of Fungi. The dynamics of hydrolysis of grass silage in terms of microbial population and process performance is yet not fully established and understood because of the complexity of different microbes associated with grass silage and with the digestion process (*87*). However, the efficient system of enzymes makes fungi a very suitable tool to achieve optimized hydrolysis of lignocellulosic substrates. Among different fungi groups, the filamentous fungi (white-rot fungi) have the ability to degrade lignin; although they are a smaller group in comparison to many other microbial groups. The oxidative nature and low substrate specificity to lignolytic enzymes make white-rot fungi efficient in biodegrading lignocellulosic materials. At commercial scale, the use of fungi can result in more cost efficient digester systems (*16*).

6.4. Laboratory Scale Up-Scaling to Commercial/Industrial Scale. Procedures adopted at the laboratory/pilot scale are different from procedures adopted in industrial-scale digesters in terms of both operational and process parameters. The major reason is economics (*8*); potential for up-scaling is often ignored at laboratory/pilot scale research. Technologies employed at the laboratory scale must incorporate the ability to up-scale to industrial/commercial scale. Two-phase processes are quite common in the scientific literature but are less ubiquitous at commercial scale. Technical limitations exist such as: leaks in the solid phase of the two-phase system due to the lack of a water seal; and leakage due to operational requirements involved in opening and closing during loading (*106*). Innovation must be applied to up-scaling and commercialization of lab-scale systems. In emptying and reloading solid-phase batch chambers the chamber should be emptied of biogas by recirculation of CO_2 from the gas combustion system, followed by insertion into the chamber of oxygen. This has a 3-fold effect: (1) the operatives are not overcome by noxious fumes when the chamber is opened; (2) the risk of explosion is eliminated; and (3) biogas losses are minimized. The range of operational and process measures adopted at laboratory/pilot scale should be in accordance with the range applicable and comparable to commercial-scale plants.

7. Conclusion

Production of biogas from grass must be seen as a holistic approach to agriculture and bioenergy production. Initially some differentiation must be observed between silage production for livestock and silage production for biodigestion. It is still important to generate a high *D*-value. However perennial rye grasses when cut young, before flowering stage, offer optimal methane potential. The first cut offers more methane than later cuts. For grass biomethane production an extra cut of silage is beneficial as compared to silage for livestock.

Laboratory work would suggest that two-phase digestion offers advantages over one-phase digestion and furthermore would suggest that hydrolysis of the grass is the rate limiting step. The importance of the integration of agronomy and biotechnology may be highlighted by noting that young grass has less lignocellulosic material than more mature grass. Biogas production of about 0.3 m^3 CH_4 kg^{-1} VS added is expected for standard digestion of grass silage. This can be improved by pretreatment. Pretreatment techniques suitable for grass silage include size reduction and thermal treatment such as liquid hot water. Codigestion with slurries can offer more stable processes, more beneficial than monodigestion of either grass or slurry.

Methods of improving biogas production from grass include for more sophisticated process control and utilization of rumen fluids to increase hydrolysis. Good grass digester design is an attempt to emulate the paunch of cattle.

Upgrading biogas to a renewable gas with 97% plus methane (termed biomethane) offers great potential as a renewable natural gas which can be distributed via the existing natural gas grid and allow countries, industries, and institutions to meet renewable energy targets particularly for transport and heat (*137*).

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Nomenclature and Abbreviations

ADF	Acid Detergent Fiber
COD	Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
DOM	Digestible Organic Matter
DP	Degree of Polymerization
DS	Dry Solid
VS	Volatile Solids
C	Carbon
D-value	Digestibility-value
FM	Fresh Matter
HMF	Hydroxy Methyl Furfural
HRT	Hydraulic Retention Time
ME	Metabolizable Energy
MSW	Municipal Solid Waste
N	Nitrogen
NDF	Neutral detergent fiber
ODM	Organic Dry Matter
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
P	Phosphorus
S	Sulfur
SBLB	Sequencing Batch Leach Bed
SCOD	Soluble Chemical Oxygen Demand
tDS	Ton Dry Solid
UASB	Upflow Anaerobic Sludge Blanket
VS	Volatile Solid
VFA	Volatile Fatty Acid
WSC	Water-Soluble Carbohydrate
XF	Crude Fiber
XL	Crude Fat
XP	Crude Protein

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