MAXIMISING THE POTENTIAL OF ANAEROBIC DIGESTION

Moving the bio-energy debate from 'fuel OR food' to 'fuel and MORE food' in a way that is economic, large scale, and sustainable.

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Abstract

Solving climate change needs new renewable technologies that can be deployed at scale economically and sustainably. These technologies must also help stabilise the grid and follow demand rather than simply generate when nature provides.

Anaerobic digestion could be part of the solution. It produces gas that can be stored and fed to engines as needed to generate electricity. However currently it is too expensive, the feedstock resource is limited, and much of the feedstock comes from energy crops that displace food. This is clearly not sustainable long term. To fulfil its potential, anaerobic digestion needs a new resource base to add to the present ones, and it needs to be much lower cost.

Part 1 of this thesis examines the costs and resource base. It concludes that the resource base could be very substantially increased by growing hyper-water-efficient plants that use the crassulacean acid metabolism on degraded and semi-arid land globally. Between 5% and 15% of this land could provide as much electrical power as natural gas. The resource could be further increased by hybridisation with solar PV. Food production need not reduce, and may even increase. To become economic, however, needs a major reduction in capital costs. This can be achieved by increasing fourfold the volumetric power density of an anaerobic digester, principally by speeding up the rate of reaction.

Part 2 of the thesis compares and contrasts digestion in ruminants with conventional anaerobic digestion technology. It concludes that ruminants perform the rate limiting step 20-30 times faster than a commercial anaerobic digester, partly as a result of adopting different mechanical and chemical strategies. The thesis identifies and explores these differences, and proposes future work to understand and quantify them, to facilitate development of a new generation of low cost Advanced Anaerobic Digestion that meets the challenging cost reduction targets set in Part 1.

Extended Abstract

Dealing with climate change needs some new thinking. It needs non-fossil fuel energy that is

a. cost competitive with coal
b. available at massive scale
c. and can be provided when the lights are turned on, not just when the sun shines.

Solar and wind meet the first two criteria, but only in limited parts of the world. They fail to meet the third criterion. Batteries will help but even if costs fall dramatically, solar/wind plus batteries will struggle to meet the cost criterion. Nuclear meets the second and third criteria, but struggles on cost and is not realistic for many parts of the world.

A technology with real promise is Anaerobic Digestion (AD). AD has the advantage over many other renewable technologies in that the biogas produced can be stored for short periods at little or no cost, and can therefore be used to supply power on demand. AD has two major problems – it is currently too expensive, and there too little available biomass globally to make much difference to global greenhouse gas emissions. This research addresses both of these topics.

AD is expensive because the tanks currently used for digestion are vast – and with them comes large sites, big pumps, large pipes etc. A ruminant such as a cow, on the other hand, is 20-30 times as fast at digesting similar feedstock to that used by AD plants. Understanding and emulating the ruminant digestive system would dramatically increase the power density of AD to create a new generation of technology, referred to here as Advanced AD (AAD). Even partial success would make AAD an economically attractive source of renewable power.

Most biomass for AD comes today from municipal solid waste (MSW) and energy crops. There is insufficient MSW to make a global impact, and energy crops that displace food are difficult to justify and costly. Crop waste is a large underutilised resource that could be used. More significant is a diverse group of plants that use the Crassulacean Acid Metabolism (CAM). These plants are ultra water efficient, so can grow on the 2.5 billion hectares of semi-arid land globally. They are not food crops, and so under-researched. Finally the AD waste can be used to grow new high protein crops as a sustainable animal feed, so increasing food production and farm incomes.

Part 1 of this thesis examines the global potential for AD, considering both cost and scale. It concludes that the combined potential of solar AAD hybrids using MSW, agricultural waste, and CAM plants grown on a modest proportion (5%-15%) of the semi-arid land is similar to global coal fired generation. It further concludes that this could be done with no negative impacts on global food production, and that, with judicious use of the by-products of AD, food production might even be increased. Even achieving 10% of this would have a major positive impact on climate change, and livelihoods in the developing world. The research into land use and the global potential of CAM crops has been published as:

Mason, P. M. *et al.* The potential of CAM crops as a globally significant bioenergy resource: moving from 'fuel or food' to 'fuel and more food'. *Energy Environ. Sci.* **8,** 2320-2329 (2015).

To achieve this economically however needs a major reduction in the capital cost of AD, and to deliver this would require a four-fold increase in the power density of an AD plant, measured on the basis of kW/m^3.

Part 2 of this thesis examines the potential to achieve this increase in power density by using ruminants as an animal model to improve understanding of the processes underlying cellulolysis, which is seen to be a key rate limiting step. It examines current knowledge in the field of AD and contrasts this with knowledge of ruminant digestive processes. It also refers to the convergent evolution of digestive processes in grazing kangaroos. Additional insights have been gathered from the construction of a stochastic model of cellulolytic biofilm growth which demonstrates how a high degree of complexity and heterogeneity can form in even a simplified biofilm model, and how the properties and environment present at the boundaries of cells in a biofilm can be extremely different to those traditionally measured in the menstruum of a reactor.

Based on this information conclusions have been drawn about the reasons that ruminants are so much more effective than AD plants at digestion, and a number of pathways have been identified that could lead to a new generation of low cost, high speed, Advanced Anaerobic Digestion (AAD).

Finally, Part 2 concludes with proposals for future work, and the design of a new generation of laboratory reactor able to emulate some of the functionality of the rumen and which might act as a platform for carrying out this research.

Preface

This research, and the thesis that describes it, is structured somewhat unconventionally. A conventional approach might involve an introduction to the topic, a literature review to determine where the gaps in knowledge lie, and then some theoretical and experimental work to plug the identified gaps. However at the heart of this thesis is a major social and economic question – how can a technology be used to its maximum effect to deliver a specific outcome?

In seeking to establish how much AD could contribute to global renewable energy, and how it could do so, the range of subjects covered has been unavoidably large; from economics and policy, through botany and agriculture, to animal nutrition and physiology, dentistry, and mathematical modelling – as well as the more narrow arena of traditional AD research. There is no single coherent body of literature that covers these topics. Consequently there is no single literature review, though Chapter 7 does review the relevant literature from the conventional AD field. Instead, the literature is described as the argument is developed.

Because the breadth of topics involved is so large, there is no easily embraced set of experiments that could be done in a reasonable timescale to supply answers. Nonetheless there is a massive body of experimental evidence available from the work of hundreds of previous researchers in each of the relevant fields. By asking new questions of old experiments, new information has been extracted from this pre-existing treasure trove of data which has allowed new, and hopefully important, conclusions to be drawn – no less than had new experiments been carried out, and much faster.

The thesis is presented in two distinct but inter-related parts. The first discusses AD as compared to other forms of bio-energy, and then examines the economics and the changes needed to make it economically sustainable. It considers the resources available, with special emphasis on the potential of CAM plants. The second part examines the theory and practice of AD, and how ruminant models might help design Advanced AD able to meet the economic challenges of Part 1. It concludes with the outline of a proposal for extensive future research to make this happen.

Acknowledgements

Firstly, I'd like to thank my supervisor, Ian Thompson, for taking on something without quite realising what he had let himself in for. I also owe a great debt to Malcolm McCulloch for encouraging me to even contemplate a D. Phil., and to be available for conversation and support whenever needed. In the labs, thanks to Duane Ager, Helen Townley and Naomi Wise, who were unstinting in their help and advice, and for introducing a complete neophyte into the world of microbiological laboratories and practice. Richard Wong made the impossible possible, by turning 26 pages of pseudo-code into near flawless programming in an inordinately short time. At Imperial College, David Stuckey gave me the benefit of his decades of experience and wisdom, and prevented me from making some egregious errors and Jem Woods provided an important sounding board for understanding and improving my approach to land use issues. At Southampton University, Charles Banks and Sonia Heaven were always available to provide ideas, support, equipment, and their wisdom and experience.

In Kenya, thanks to Barney Gasston and Live Energies for giving me unstinted access to their trial site and data. At the Centre for Dairy Research at Reading University, Chris Kent-Reynolds and David Humphries opened their facilities to me, and let me dig around in Daisy, the fistulated cow. At Kew Botanical Gardens, Kathy Willis gave me the confidence to be bold about thinking about CAM plants, and Andrew Smith spent many hours explaining to me the delights of CAM. Thanks also to Andrew, and to Kat Glover, for their endless patience in proof reading and correction; Andrew of the paper we jointly submitted, and Kat of both the paper and this thesis.

Kat is owed a special vote of thanks in leading me down the true path of decent referencing, by challenging every assertion and making sure the references were robust.

At home I have to thank my endlessly patient wife, Caroline, for feeding and watering everyone who turned up at home to talk about AD, and for letting me abandon her as a D. Phil widow for hours and days at a time.

Finally, I acknowledge a debt to Tropical Power Ltd, whose shareholders had faith in my ideas and funded this program.

List of Abbreviations

AAD	Advanced anaerobic digestion
AD	Anaerobic digestion
b.p.	Before present
CAM	Crassulacean acid metabolism
CSTR	Continuously stirred tank reactor
DMT	Dry metric tonne
EIA	US Energy Information Administration
FAO	Food and Agricultural Organisation of the United Nations
GHG	Greenhouse gas
GW	Gigawatts
IRR	Internal rate of return
kW	Kilowatt
kWh	Kilowatt hour
kWh(e)	Kilowatt hour of electrical power
MC	Moisture content
MDa	Megadaltons
MJ	Megajoule
mM	milliMolar
MMkcal	Million kilocalories
MSW	Municipal solid waste
MTOE	Million tonnes of oil equivalent
MW	Megawatts
MWh	Megawatt hours
NOAA	National Oceanic and Atmospheric Administration of the USA
PEG	Polyethylene glycol
PNAS	Proceedings of the National Academy of Sciences
PV	Photovoltaic
PWh	Petawatt hours

TPES	Total primary energy supply
TWh	Terawatt hours
VFA	Volatile fatty acid
VS	Volatile solids
W	Watts

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PART 1

ACHIEVING THE POTENTIAL OF ANAEROBIC DIGESTION

Part 1 of this dissertation considers the potential of Anaerobic Digestion (AD) to play a major role in mitigating anthropogenic climate change. It examines the economics of AD, and the potential scale of its contribution to renewable energy generation. Crucially, it also considers the sustainability of the technology and its potential to move the bio-energy debate from "fuel or food" to "fuel and more food".

It concludes by setting out a target for improving the power density of a commercial AD plant to a level that would make it competitive with fossil fuel generated power, and also demonstrating how, with the use of novel hyper-water-efficient crops, AD could rival or exceed the generating capacity of many conventional sources of power.

The research presented here on the potential of CAM crops as a globally significant sustainable source of bioenergy was published in the Royal Society of Chemistry's journal, 'Energy and Environmental Science' in July 2015.[1]

Chapter 1 INTRODUCTION

1.1 THE CHALLENGE OF RENEWABLE TECHNOLOGY – A SYSTEMS APPROACH

In 1992 the UN Conference on Environment and Development, otherwise known as the Earth Summit, established the UN Framework Convention on Climate Change. This was followed by the Kyoto Protocol in 1997, which was ratified by a quorum of countries and came into force in 2005. The purpose of this process was to start shifting the world away from fossil fuels to a low greenhouse gas (GHG) development path that would ensure a sustainable climate for future generations. However, after over 20 years of discussion, and nearly ten years of binding targets, Figure 1 demonstrates that there is no clear signal in the global emissions data that indicates any degree of emission reduction has take place, and that the rate of increase in concentration actually accelerated in each decade, with the sole exception of the years from 1990 to 2000 when the collapse of the Soviet Union resulted in a major reduction of emissions in Eastern Europe and Russia.

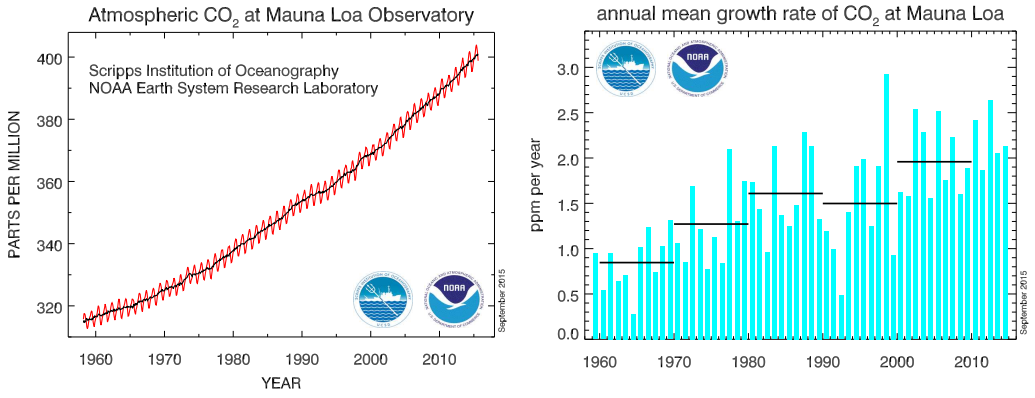


Figure 1 - Atmospheric CO_2 concentrations and rate of change of CO_2 concentration at Mauna Loa since 1958 (reprinted from NOAA).[2] The **rate of growth** of CO_2 concentrations (right hand graph) shows increases every decade save the 1990s following the collapse of the Soviet empire. However even in this decade the actual growth was positive.

This begs the question "Why has there been no change?". Probably the most persuasive answer to this question is that the technology to replace fossil fuels is not yet right. There are several components to 'not right'. Clearly cost is a major issue – with democracies by and large unwilling to impose high costs of energy on their voters.

Cost alone though is not enough to explain the lack of progress - in some areas costs are already beginning to compete with fossil fuels.[3] A clear example of the continuation of fossil fuels in the face of cheaper renewable energy is the large amount of electricity still generated using diesel or fuel oil. According to the IEA in 2011 4.8% of the world's electricity was generated with oil.[4] Of this a substantial proportion will have been generated in reciprocating engines with diesel or fuel oil. At current (2014) costs of around $1.00/litre, with an energy content of around 10kWh/litre, and typical generator efficiency of around 40%, the fuel cost alone of diesel generation is around $25cts/kWh excluding capital, operation and maintenance. For comparison, the feed in tariff for solar PV in Kenya is $0.12/kWh and there is no shortage of willing developers at this price.[5,6] Ministry of Energy officials cite 750MW of projects in the pipeline yet Kenya still uses diesel and fuel oil to generate power.[6] One reason is simply that there is no technical way to incorporate the solar projects that developers wish to develop into the grid without completely re-thinking the current operating philosophy – in particular in relation to hydro power. The entire grid is only 1.77GW with an average supply of 0.84GW.[7] Injecting such a large quantity of unreliable energy would be impossible in the absence of major re-engineering of the grid and incorporation of storage and new despatch and demand-side management strategies.

Kenya is not alone in needing to take a new systems approach to renewable energy. Spain recently introduced a retrospective tax or charge on solar installations to account for the costs of backup power borne by the rest of the generating system which seem not to have been foreseen when policy was implemented.[8] Likewise Germany and France suffered the consequences of inadequate systems planning when the electricity wholesale market faced negative prices in the summer of 2013 as a result of excessive solar generation, and Germany faced negative prices in the winter of 2013 with excessive wind generation.[9,10] Such badly behaved markets send strong signals to other countries not to install more than minor amounts of intermittent renewables. In both Hawaii and Queensland energy planners are seeking to limit excessive PV power.[11]

Thus it must be concluded that a second objection to the deep penetration of renewable energy into current energy systems is a lack of flexibility and convenience. Electricity doesn't store well, and power is needed when customers turn the light switch on, not when nature chooses to provide it.

A third pre-requisite for renewable energy to make the contribution needed to materially reduce GHG emissions is scale. Examples of renewable technologies that are cost effective and convenient but too minor scale to have a material impact on global greenhouse gas emissions are bio-diesel from cooking oils, and electricity from landfill gas. Both of these are tiny players in the renewable energy space, simply because the resource base is inadequate.

Finally, even for technology that has convenience, cost and scale, there is the challenge of sustainability. For example, both bio-ethanol and bio-diesel face severe constraints on their production as a result of social and environmental issues that they create as much as a lack of physical resource or excessive cost.[12,13] Bio-ethanol may overcome these to a degree if future developments of cellulosic ethanol remove the competition for food by using cereal straws, but whether this can be made economically competitive with gasoline is uncertain. Biodiesel, derived as it is from plant oils, faces a much greater challenge. Fargione et al. point out the negative environmental consequences of biodiesel production even though the technology offers the cost and convenience needed.[14]

Thus, for any renewable energy technology to make a significant contribution to global GHG reduction it needs to fulfil the following requirements.

a. It must be the best way to use the resource available.
b. It must be able to compete on cost terms with fossil fuel alternatives.
c. It must be usable in existing energy systems at high levels of penetration.
d. It must be of a sufficient scale to have a material impact.
e. It must be sustainable at scale, and neither compete with food production, nor make a material impact on valuable ecosystems such as forests.

If AD is to make a material impact and help bend the curve of ever rising emissions it must be possible to demonstrate that it can, or could, meet all these criteria. The following sections address each of these questions in turn – to establish where AD already meets the criteria, and where additional work must be done.

1.2 The Best Use of Biomass Resource – Solid, Liquid or Gas?

A key question to address before launching into an in-depth analysis of Anaerobic Digestion is – "Is AD the right energy conversion technology for biomass?"

Biomass, can, of course, also be used as a solid fuel (wood, charcoal), a liquid fuel (ethanol, biodiesel), or gas for electricity generation, so consideration needs to be given to the alternative energy conversion pathways to determine whether AD is likely to make a major contribution to GHG reductions in the long term or whether it will be superseded or out-competed by other technologies. Whilst it is not possible to be certain about future technology developments, the different conversion pathways have quite different characteristics and outcomes, which largely set their future potential. This chapter considers the alternatives to AD in order to assess the likelihood that it will be a dominant technology for biomass use in the long term.

Bioenergy from solid fuels

There is little cross-over between biomass as a solid fuel, and AD. Woody biomass is a major fuel in much of the world, either as firewood or charcoal, but it is not ideal for AD because of the high lignin content. Some crop waste is briquetted for use as cooking fuel, but it is not a major consumer of biomass. Cooking is a notoriously inefficient user of biomass with serious health and environmental consequences, and once gas or electricity are available one might anticipate a rapid switch into the new fuels. Thus given the extent of deforestation caused by wood and charcoal use for fuel, it seems likely that uses for solid biomass will largely become confined to power stations burning wood for power. These need low moisture content, and prefer low ash fuels such as wood rather than the problematic high ash, low ash melting temperature, grasses.

It is also worth noting that AD might actually increase the availability of sustainable solid fuels. The undigested solids that are produced by an AD plant should, in principle, make good charcoal. This is a subject that is discussed briefly in Chapter 4 but merits further research.

Ethanol or Biogas

A greater potential for competition exists between AD and ethanol as alternative uses for crop wastes or any wet biomass. Ethanol is seen as an ideal fuel for transport and some uses such as

cooking. It is clean, easy to transport and store, and can be used in existing cars and cook-stoves. Consequently considerable effort is being directed to converting agricultural cellulose into ethanol as a high value form of renewable energy and much of the discussion about bio-energy in the literature is framed around the assumption that biomass will be converted to liquid rather than gas.[15]

There are two potential routes to ethanol.

1) The first is through non-cellulosic material such as sugars, largely from sugar cane (*saccharum officinarum*), or from starch, largely corn (*zea maïs*). This route suffers from the problem of competition for food. Either the feedstock is food itself or in the case of sugar cane it occupies land and consumes water that could be used for growing food. In a future world of over 10 billion people seeking a Western meat-rich diet this is unlikely to be an acceptable use of land and water.

   World total primary energy supply (TPES) in 2013 was 13,113 million tonnes of oil equivalent (MTOE).[4] Current global bioethanol production is approximately 23 x 10^9 US Gallons/year.[16] This is equivalent to 87 x 10^6 m^3 of ethanol, or 44 MTOE. Given that ethanol is already widely subsidised and mandated, the fact that it only provides 0.33% of TPES, together with the issues of food competition, suggest that ethanol from sugar or starch has reached an economic or resource constraint.[17]

2) The second route is from cellulose. This can either come from trees, or from the straws of cereal crops. Cellulose is, however, difficult to break down. Both AD and ethanol production require hydrolysis of cellulose and hemicellulose with enzymes derived from bacteria, protozoa or fungi, to produce simple sugars. In the production of ethanol this creates two problems not faced by AD:
   a) The first is that the organisms that express the cellulolytic enzymes do so in order to consume the sugars that are produced from hydrolysis. Thus to retain the sugars so that they can be fermented to ethanol by yeasts, the enzymes have to be produced outside the reactor vessels and added to the reactors independently of the organisms that produce them. This compares with the production of methane from AD, where the

enzyme producing organisms live in the reactors and consume the sugars themselves to produce acetate and other VFAs, which in turn are converted to methane. This means that enzyme production for AD is essentially free.

b) The second is that ethanol is soluble in water, so capital equipment and energy are needed to separate and purify it to become a usable fuel. AD, on the other hand, produces methane which bubbles off as a gas so that no energy or additional equipment is required for separation. The methane is mixed with CO_2 but this has minimal impact on its use as a power generation fuel.

Thus it is likely that cellulosic ethanol production will always be more expensive per GJ than methane production when using the same feedstocks. The question that remains is the relative value of the products – both financial and in energy terms.

THE COMPARATIVE ENERGY VALUE OF ETHANOL AND BIO-METHANE

The energy yield from lignocellulosic ethanol is around 227 litres/tonne of corn stover.[18] The comparable yield of biomethane is around 360 litres/kg volatile solids (VS) with a VS concentration of 91.32%, giving net yields of 327 litres/kg of dry matter.[19] Table 1 shows the comparative energy yields of cellulosic ethanol and biomethane from a tonne of corn stover.

Table 1 - Energy yields of ethanol and biomethane, showing energy yields of the primary products and ignoring either parasitic electricity costs or surplus electricity generation.

	Ethanol	**Methane**	
Feedstock	1,000.00	1,000.00	kg
Gross Yield	227.00		litres
		327.00	m3
Density	0.79		kg/litre
		0.66	Kg/m^3
Mass	179.10	215.82	kg
CV	28.90	50.00	MJ/kg
Gross energy yield	**5,176.08**	**10,791.00**	**MJ/t**

This data is something of a simplification because it ignores secondary energy flows. Thus an AD plant built using current technology that produces electricity from biogas uses around 7%-10% of electricity generated to operate the plant, and also a proportion of the heat from the engines is

similarly used. Additional spare heat which may have process or other uses is ignored, as is the energy value of the solid waste that could be turned into charcoal or fuel briquettes.

Conversely a well designed cellulosic ethanol plant is capable of producing electricity from the un-hydrolysed matter sufficient to power itself and leave a small amount for export. A theoretical analysis by National Renewable Energy Laboratory in the USA of cellulosic ethanol plant economics puts this at 16 MMkcal/h for a 100 tonne/hr. plant (670 MJ/t, or 190 kWh/t).[20]

Table 2 compares the net usable energy available from the two routes – assuming in both cases conversion from chemical to mechanical or electrical energy at around 40%. Note that the energy used in production of the enzymes is not included in the analysis. A reasonable approximation therefore is that an AD plant can extract almost 50% more usable energy from corn stover than an equivalent ethanol production plant.

Table 2 - Useful Energy Yields Compared, taking the data from Table 1 and adjusting for power generation and parasitic power consumption. Although this adjustment favours ethanol, the production of CH_4 still shows a significant energy production advantage per tonne of feedstock.

	Ethanol	Methane	
Gross energy yield	**5,176.08**	**10,791.00**	**MJ/t**
Useful energy at 40% conversion η	2,070.43	4,316.40	MJ/t
Surplus/parasitic electricity	670.00	(302.15)	MJ/t
Net useful energy	**2,740.43**	**4,014.25**	**MJ/t**
Relative yield	**100%**	**146%**	

COMPARATIVE UTILITY OF CELLULOSIC ETHANOL AND BIOMETHANE.

Neither ethanol nor biogas are, in themselves directly useful; both have to be converted to something else. In the case of bio-ethanol this is usually in a mobile internal combustion engine such as a car or truck, and in the case of biogas it is usually an internal combustion engine to generate electricity. Thus both of these face similar thermodynamic constraints.

Ethanol from cellulose could of course be converted in stationary plant to electricity, but it would be pointless given the high cost of production and the low energy yield. The great advantage of ethanol is its convenience and transportability – which is why its greatest value comes as a transport fuel. Thus a better measure of the relative utility of ethanol and electricity comes from a comparison of the two forms of energy in providing transport. A study in Science concluded

that cellulose to electricity via biogas produced over 80% more transport miles than cellulosic ethanol per unit of feedstock.[21] This is consistent with the analysis above.

Another aspect that must be considered is need. For most of the developing world, which in effect means most of the world, liquid fuels are not necessarily the highest priority. For them the priority is electricity – for light, refrigeration, entertainment, communication, and commerce. Although there may be limited use of electricity in many places today this often reflects lack of affordable supply rather than lack of demand.

Conversely, improvements in efficiency in vehicles together with growing electrification means that road transport fuel use is likely to fall over the coming years. Figure 2 shows forecast CO_2 emissions from road transport for England, which provide a direct proxy for fuel use. This shows quite substantial drops in spite of population growth.

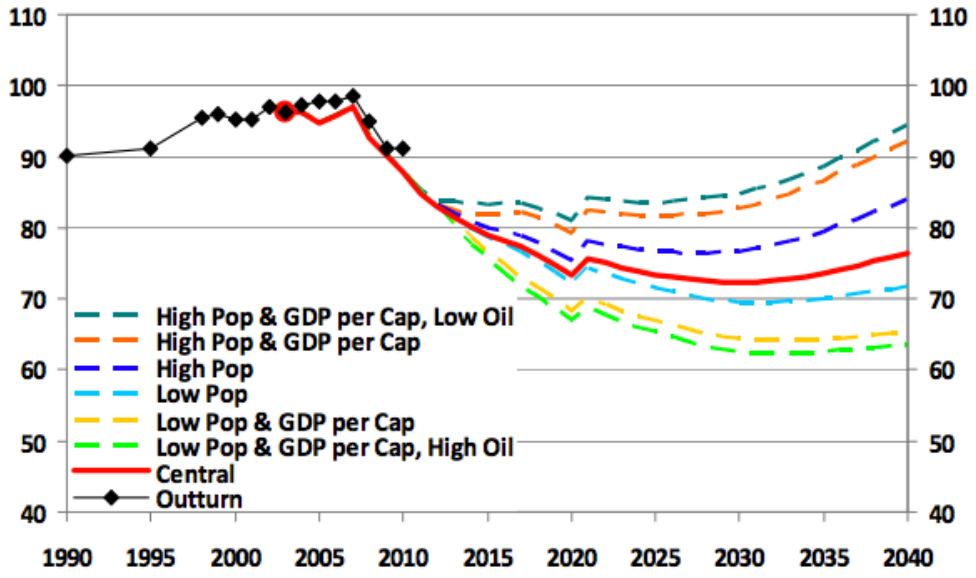


Figure 2 - Road transport CO_2 emissions forecast for England.[22] CO_2 emissions provide a direct proxy for fossil fuel use. (*Reprinted from UK Dept. Of Transport*).

By the time many of the developing economies achieve near universal car ownership it is probable that the motor industry will have converted to efficient plug-in hybrids or fully electric vehicles. This will add to the need for electricity as much or more than it adds to the need for liquid fuels. According to a report by the IEA, considering only companies and countries in the Electric Vehicle Initiative, stocks of electric vehicles in one form or another are targeted to reach 20 million by 2020 (Figure 3).[23] Given that the EVI includes 8 out of 10 of the world's largest car manufacturers this number has some credibility.

SUMMARY

Woody feedstocks with high lignin content are most likely to end up as solid fuel, or as feedstock for cellulosic ethanol plants. On the other hand, if a feedstock is suitable for anaerobic digestion it is likely, in the longer term, to be converted to electricity via biogas. It will deliver greater energy output per hectare of land, and serve the needs of society better.

1.3 COMPETING WITH FOSSIL FUEL – ESTABLISHING COST TARGETS

The default fuel for most electricity generation is coal, though gas is becoming increasingly common. Competing on cost with existing coal fired plant that has been fully paid for will always be difficult – but as the world's economies grow and developing countries become more industrialised and richer, it is new coal fired plants which will provide the benchmark for competition.

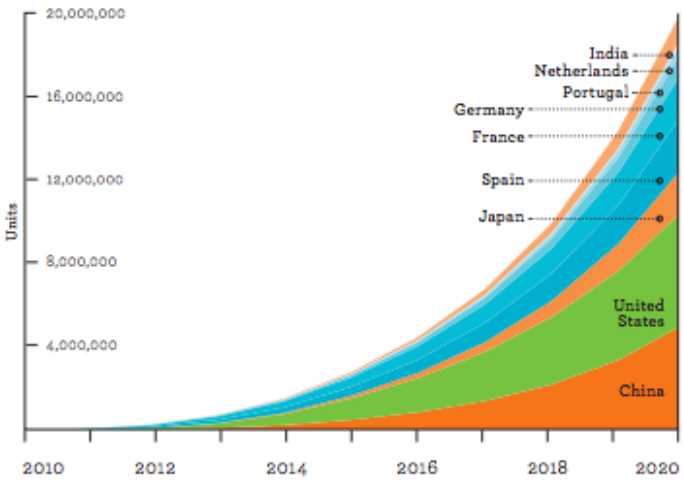


Figure 3 - Electric vehicle stock forecast (*reprinted from the IEA*)

The US Energy Information Agency Annual Energy Outlook publishes the anticipated costs of electricity from new generation 5 years ahead of the date of publication. This data provides a useful guide to fossil fuel energy costs, but with certain caveats. Firstly the data is based on North American costs of capital – assumed to be 6.5% in 2014.[24] This is probably unrealistically

low for much of the world outside North America and parts of the EU. Secondly, the calculations compensate for this somewhat by increasing the cost of capital for carbon intensive industries by 3%, to bring it to an average cost of capital of around 9.5%. Table 3 shows the latest estimates. In these data the cost of electricity from coal fired conventional power stations averages around $96/MWh.

Table 3 - US EIA Electricity Cost Forecasts 2014

	Capacity factor (%)	Levelized capital cost	Total system LCOE		
Coal fired			**Low**	**Mean**	**High**
Conventional Coal	85%	$60.00	$87.00	$95.60	$114.40
Integrated Coal-Gasification Combined Cycle (IGCC)	85%	$76.10	$106.40	$115.90	$131.50
IGCC with CCS	85%	$97.80	$137.30	$147.40	$163.30
Natural Gas-fired					
Conventional Combined Cycle	87%	$14.30	$61.10	$66.30	$75.80
Advanced Combined Cycle	87%	$15.70	$59.60	$64.40	$73.60
Advanced CC with CCS	87%	$30.30	$85.50	$91.30	$105.00
Conventional Combustion Turbine	30%	$40.20	$106.00	$128.40	$149.40
Advanced Combustion Turbine	30%	$27.30	$96.90	$103.80	$119.80

Individual AD plants are unlikely to ever reach sizes greater than a few MW, simply because of the mass of material that needs to be collected and the challenge of co-ordinating farmers where no one farm can provide the full feedstock. Although there may be some economies of scale with larger AD plants these become very minor as the plants exceed 3MW, and the benefits are outweighed by the challenges of feedstock supply. Thus AD businesses will always be smaller than coal fired power stations, and small businesses face higher costs of capital in general than large ones. Therefore, for the purposes of this research, $100/MWh at 15% -20% cost of capital is taken as the target that AD must achieve if it is to compete with coal – although a straight, like-for-like comparison would be to use a 10% or even lower cost of capital.

Chapter 2 THE RESOURCE BASE

This chapter examines the potential resource from conventional sources, namely municipal waste and traditional crop residues that could be used as AD feedstock, and then it considers in detail a novel group of plants that might add significantly to the potential of AD. Finally, it examines how the AD resource base might be amplified by hybridisation with solar PV.

2.1 THE CONVENTIONAL RESOURCE BASE

MUNICIPAL WASTE

Anaerobic digestion has been seen for many years as a waste treatment technology – and it continues to be used as such. Municipal Solid Waste (MSW) is widely seen as an important potential feedstock for AD. Nonetheless the data tell a different story.

A 2012 analysis of global MSW carried out by the World Bank estimates that, globally, around 3.5 million tonnes/day of urban MSW is produced, of which 46% is organic (excluding paper which will probably go to recycling).[25] The moisture content of this material is likely to be of the order of 50%.[26] This gives a total estimated generating potential of 0.3PWh/year, as demonstrated in Table 4.

Table 4 - Power generation potential of Municipal Solid Waste (MSW)

	2012	
Global Waste Tonnage	3,532,000	tonnes/day
Organic fraction	46%	
Moisture content	50%	
Ash	13.30%	
Dry tonnage	704,316	
Energy production	1.2	MWh/tonne
Power output	35,216	MW
	308	**TWh/yr**

AGRICULTURAL WASTE.

Regardless of economics, if the planet is to support 11 billion people sustainably at some stage in the future, with a Western lifestyle and diet, diverting quality agricultural land to energy seems

unlikely to be a wise choice. A better approach may be to use agricultural waste. One study, by Lal, estimates global cereal crop residues at approximately 2.8 billion dry tonnes/year, with approximately 1 billion tonnes from other crops, bringing the total to around 3.8 billion dry tonnes/year. [27]

These total tonnages fail to reflect the reality of what is available at an acceptable cost. The issue is that the economies of scale at the plant level favour larger and larger plants, but this creates dis-economies of scale in collecting and securing the resource. These dis-economies are, in part, transport related, but a more serious problem is the need to secure the long term commitment and co-operation of more than one farm – where owners are small businesses or individuals with varying needs and variable finances. This sets the scene for needing smaller units capable of operating on single farms or a small group of farms, rather than needing tens of thousands of tonnes a year. Perhaps, at most, 50% of the potential waste could be captured and converted to electricity. At 1.2 MWh/tonne this would generate 1.7 PWh/annum, equivalent to 190 GW on a continuous basis.

Note on conversion of biogas to electricity

Typically crop biomass produces 300-360m³/dry tonne of CH_4, equivalent to 1.3-1.56 MWh/dry tonne. Actual figures depend on the crop, its ash and lignin contents, and whether digestion is thermophilic or mesophilic.

Given the uncertainties, a mid range figure of 1.2MWh per dry tonne of biomass is used throughout this dissertation unless otherwise specified. The calculations below put this in context.

CV of methane	*39 MJ/m³*
Efficiency of engine	*40%*
Electricity/m³ CH_4	*4.33 kWh/m³*
240m³/dry tonne =	*1.04 MWh/t*
300m³/dry tonne =	*1.30 MWh/t*
360m³/dry tonne =	*1.56 MWh/t*

Lal points out, though, that crop removal has consequences for soil health. One of the characteristics of AD is that the current generation of AD plants produce around 15% solid waste from the feedstock input, distributed between the liquid digestate, where it appears as a fine suspension or colloid, and a solid digestate. This material is composed of the recalcitrant fractions of the feedstock – presumably largely lignin, together with all the nutrients present in the original crop. This has distinct similarities with compost, and may well provide much of the same benefits as leaving crop waste on the soil provided it is returned to the soil as fertiliser. Although the tonnage is lower than would be achieved by leaving raw crop waste, the recalcitrant nature of the material means that it will last much longer.

2.2 THE UNCONVENTIONAL RESOURCE BASE

In order for bioenergy to make a real difference to global climate change, a new source of biomass is needed. However, if it must be sustainable at scale, and neither compete with food production nor make a material impact on natural ecosystems such as forests, this has to be produced on land for which there is little ecological or economic competition. The only places where such land is available in any quantity are the areas where soils are poor, and rainfall limited and or irregular. Estimates vary, but this research argues that there may be somewhere between 2 and 5 billion ha that could theoretically be available, though undoubtedly only a modest proportion of this will be usable, for ecological or other reasons. Much of it may also have competitive use as pasture, albeit possibly of low productivity.

There is a group of plants that have evolved in these semi-arid areas and are adapted to conditions of intermittent water availability: these plants use a particular photosynthetic pathway known as crassulacean acid metabolism (CAM).

Because CAM plants are characteristic of seasonally arid environments where their productivity is severely limited by restricted water availability, CAM is often perceived as a relatively unimportant metabolic pathway compared to the better known C3 and C4 photosynthetic pathways found in all of our staple crop plants. Nonetheless, there may be as many as 16 000 species of plants that use the CAM pathway of photosynthesis, and some of these have been shown to achieve high productivities under more favourable growing conditions.[28–30] The potential that derives from their unique metabolism seems to be severely under-studied and under-exploited.

The potential of CAM plants for bioethanol use has been identified by a number of authors, but there has been scant academic interest to date in CAM plants as feedstock for anaerobic digestion (AD) to biogas for electricity. [31–40] Nonetheless, a significant body of literature exists that relates to the use of CAM plants, and *Opuntia* in particular, as supplementary animal fodder, and the parallels between ruminant and commercial anaerobic digestion are sufficiently strong as to allow the research done in animal nutrition to be applicable to the energy field. [41–45]

There is likely to be only limited competition between biomass grown on semi-arid land and food production. However, one consequence of growing CAM plants is that they accumulate large quantities of water and nutrients – both of which are conserved in AD, and can be reused once digestion is completed. This leads to the possibility of growing high-value crops from the waste products of AD that would lead to an overall increase in the value of food produced from the land.

In other areas where arable farming is possible but marginal, the addition of CAM crops to a farmer's portfolio could lead to enhanced income security, and more capital deployment in agricultural infrastructure. This has the potential to increase food production several fold by improving the quality of agriculture.

WATER USE EFFICIENCY

The CAM pathway of photosynthesis enables the temporal separation of CO2 fixation and assimilation during the day–night cycle. This allows plants to absorb CO2 from the atmosphere during the night when air temperatures are lower – so reducing evaporative losses – and to metabolise the stored carbon during the day when solar energy is available. Although this is the defining characteristic of CAM plants, as a group they exhibit a significant degree of plasticity in their photosynthetic behaviour, and many can supplement their nocturnal CO2 fixation with diurnal CO2 uptake when water availability is high.[31] This flexibility makes them attractive candidates for use as energy crops in semi-arid and variable rainfall areas.

The high water-use efficiency of the CAM pathway of photosynthesis is striking.[31] CAM plants are reported to assimilate 4–10 mmol CO_2 per mol H_2O, compared to 1–2 mmol CO_2 per mol H_2O for C4 plants, and as little as 0.5–1.5 mmol CO_2 per mol H_2O for C3 plants. Further data on the water-use efficiency of *Opuntia* species from field studies and a collation of research by the FAO supports these conclusions. [30,41]

It is this very high water-use efficiency under semi-arid conditions that distinguishes CAM plants as potential energy crops at a global scale. Of all the potential sources of biomass, they have the greatest land area available to them with the least competition.

CAM PLANT COMPOSITION

Few of the 16 000 or so species of CAM plants have been studied in detail, so records of their chemical composition are comparatively sparse. From an energy perspective, only one detail is really important – namely the lignin content. Lignin is well known to impair AD, both by physically occluding more digestible material and also by adsorbing and de-activating hydrolytic enzymes, and lignin content has been shown to be the best predictor of ultimate biogas potential.[46–50] The low transpiration rates of CAM plants in general leads to low xylem tensions in the vascular system, and thus reduces the need for structural lignin for support on the scale seen in the massively woody stems of trees.[31,32] Nefzaoui and Ben Salem summarize the composition of several *Opuntia* species, citing lignin content in the range of 2.9% to 4.8% of dry mass.[41] Yang et al. analysed the composition of one-year-old cladodes of O. ficus-indica.[51] They separated the cladodes into a juice and a bagasse, and determined the lignin content of the bagasse to be 12.3 ± 1.1%; given that the juice contained slightly over 62% of the total dry matter, this equates to a total lignin content of around 4.7%. Cushman et al. also reviewed the lignin content of six leaf-succulent agave species and found them to be in a similar range.[52] These low lignin levels in CAM plants should thus make them favourable as a feedstock for AD.

POSSIBLE CANDIDATE SPECIES

There are numerous species of *Opuntia* and *Euphorbia* that have yet to be investigated in any systematic way, either for their agronomy or their energy potential. For example, Lopéz-García et al. list 17 species of *Opuntia* plus four varieties of *O. ficus-indica* which have been considered for animal forage in dry regions, and appear also to be suitable for AD.[43] Likewise, Calvin highlighted the potential of *E. lactaea* and *E. lathyrus*, but little seems to have been done to quantify their potential as energy crops.[37,53–55]

Most of the historic energy interest in CAM has been for bioethanol production, with a particular focus on agaves.[31–33,36] Although agaves are relatively well studied and show many favourable traits for AD, they are problematic in that they cannot be coppiced or mechanically harvested.[56] Planting, harvesting and replanting of agaves are also highly labour-intensive activities. However, if energy crops are to be grown in areas where there is little or no competition with

food, then almost by definition they will be growing in areas with little or no population, and thus labour will be scarce. This trend is exacerbated by the tendency of people living in remote semi-arid areas to migrate to towns, and away from backbreaking tasks such as manual harvesting of agaves, as soon as circumstances permit. This creates challenges for the use of agave as an energy crop.

The need for a low labour input dictates a high level of harvest mechanisation. Forage harvesting is a well established agricultural technology, and forage harvesters are widely used for both agricultural crops (e.g. maize silage) and woody energy crops (e.g. willow coppice). This leads to the conclusion that a practical CAM energy crop needs to be one that can be forage harvested.

A second desirable characteristic is that the crop should coppice well. Planting from seed is expensive, and planting from cuttings or other propagules requires labour. Coppice crops, on the other hand, need no replanting. The other great benefit of coppicing is that the crop retains its roots from one cycle to the next. This maximises growth rate and the ability to harvest water, whilst minimising soil and nutrient loss – all key features in a semi-arid environment. Indeed, in addition to their superior water-use efficiency, CAM plants are also predicted to be more economical than C3 plants in their use of nitrogen on account of their mode of photosynthetic carbon assimilation[31].

A third desirable characteristic is that the plant should have a low water content. A full economic model has been constructed of a CAM plantation providing fuel to a 1.8 MW AD plant in order to assess the sensitivity of the operation to both crop yield and moisture content (MC). This is discussed in Chapter 3 in detail. It shows that, whilst yield is slightly important, moisture content is a far more important economic characteristic. Even small increases in MC increase very significantly the mass that must be harvested and hauled to deliver a tonne of dry biomass. Many CAM plants can have MC well over 90%. The difference between 85% and 95% MC (i.e. 15% and 5% dry mass to fresh mass ratio, respectively) is a 300% increase in tonnage that must be harvested for the same potential energy yield.

Thus, the ideal CAM energy crop will be a plant that can be harvested mechanically by forage harvester, coppices well over many years, reproduces easily and rapidly, and has a low water content. .

Opuntia species

Species of *Opuntia* cacti are prime candidates for energy crops, as they are easy to propagate, coppice well, make maximal use of photosynthetically active radiation (PAR) and are easy to digest anaerobically.[30,41,45,51,52,57–61] Figure 4 illustrates the results of a trial crop propagated from single cladodes at 10 months old, in Laikipia in Northern Kenya.

There is one non-technical problem for *Opuntia* species, namely widespread perception that they are hostile invasive species outside their native habitats in the Neotropics (tropical and subtropical America). Certainly Australia and South Africa have faced challenges in removing them.[62–65] There are many potential solutions to this issue, but the simplest may be to use spineless varieties such as *O. ficus-indica* var. *inermis*, or *O. ellisiana*. These are likely to be subject to substantial grazing pressure outside any plantation by either wild herbivores or cattle, sheep and goats – especially in semi-arid areas where they provide both food and water.[66] In addition, biological control with the *Cactoblastis* moth has proved widely successful.[62]

Figure 4 - Ten-month-old *Opuntia ficus-indica* in Laikipia, Kenya (photo credit George Francis).

Notwithstanding this, *Opuntia* species seem to be an attractive potential crop that could significantly increase the AD resource. There is, furthermore, significant potential for

improvement across a range of key traits, and a concerted research effort into the agronomy and opportunities for improvement would be likely to pay large dividends.[44]

Euphorbia tirucalli

Another species of considerable interest for AD is *Euphorbia tirucalli* from the family Euphorbiaceae. *E. tirucalli* is an African native plant widely distributed in Africa and Asia, and used as an ornamental in many other parts of the world. Figure 5 shows *E. tirucalli* grown in Laikipia at planting density up to 266 000 plants ha^{-1}. In Africa it is used both medicinally and as a stock-proof fence. It has also been recognised as a potentially valuable source of a range of chemicals including possible liquid fuels.[35,37,53,55]

Many *Euphorbia* spp. also use CAM photosynthesis in their green stems, but during periods of rainfall produce thin, deciduous leaves that utilize the standard C3 mode of photosynthesis – enabling them to make the most of intermittent moisture availability.[35]

Figure 5 - *Euphorbia tirucalli* under test in Laikipia, Kenya (photo credit George Francis).

E. tirucalli produces copious quantities of latex, which is recognised as an irritant and is probably mildly toxic to animals as few are known to graze it; the latex is used as a biocide and avicide in some places.[67] Although long-term testing is needed to determine whether there are any issues with anaerobic digestion as a result of the build-up of toxic products caused by digestate cycling, some limited testing has been carried out. This shows that, in the short-term and at laboratory scale, the plant digests well and suggests that if there are any long-term issues these may be overcome by process optimisation.[37,68]

ECONOMIC POTENTIAL

Although this is an area in which further research is needed, there is sufficient data available to make preliminary estimates. The major factors that determine the economic potential of a crop for AD are the moisture content and the growth rate.

Moisture content

The summary of Lopéz-García et al. [43] of *Opuntia* properties is helpful in showing a consistent picture across the genus of high water content, averaging around 88% (Figure 6), but it also reveals considerable variation between species and even within species sampled by different investigators in different places and at different times. For example, *O. imbricata* dry matter content varies between 10.4% and 17.7% – itself a variation of 70%. This suggests that whilst estimates of the global economic resource can be made, local conditions and species choices will have a very large influence on actual outcomes which will be difficult to forecast.

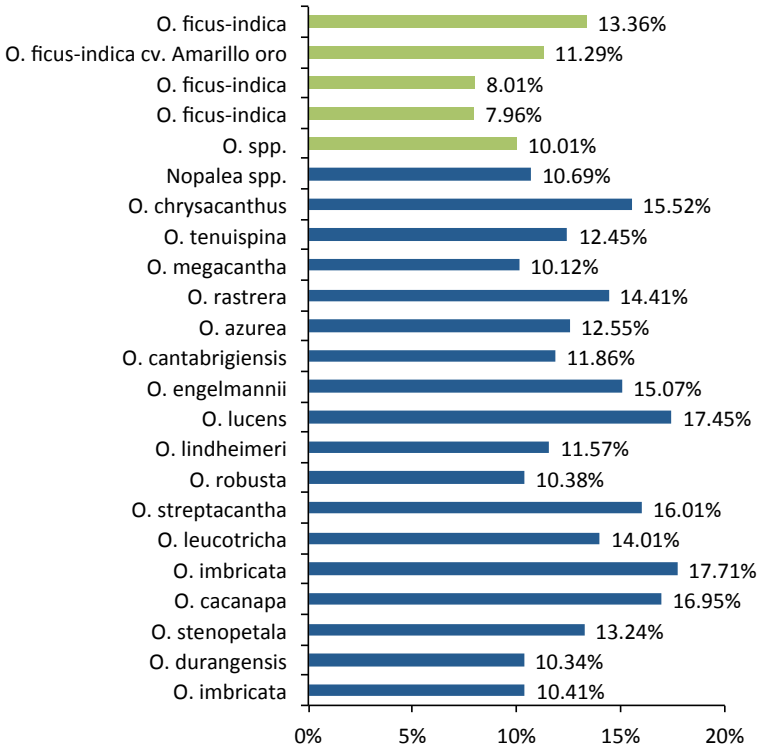


Figure 6 – Dry matter content from a range of *Opuntia* studies adapted from FAO [43]

Although the data are limited, *Euphorbia tirucalli* currently seems more promising as a biomass feedstock than *Opuntia ficus-indica*. A limited trial in Kenya reports MCs of 83%; Hastilestari et al. investigated *E. tirucalli* at a range of different soil volumetric water contents (VWC) and found

a range of MC of 80% to 87%.[69,70] The lower soil VWC corresponded to lower plant growth rates, but also materially lower MCs, so compensating for lower yield with lower harvesting and haulage costs.

Growth rate

Four published data sets provide estimates of yield as a function of rainfall for *Opuntia ficus-indica*:

d. Le Houérou gives an absolute minimum for rain-fed crop establishment of 200 mm in sandy soils of North Africa, except in the far West where 150 mm is possible.[66]
e. Nefzaoui and Ben Salem provide estimates of fresh matter yield per hectare per year in Tunisia as a function of rainfall.[41] In the absence of better data, the following analysis assumes the arithmetic average *O. ficus-indica* organic matter content quoted in Figure 4, namely 10.2%.
f. Dubeux et al. cite data from four sites in the semi-arid region of Brazil.[58] Their data are for two planting densities – one low and the other high. The lower planting density can be disregarded because it does not reflect the potential that can be achieved with full interception of available PAR. At higher densities the annual growth rate is higher, though one might expect this to show diminishing returns once most of the PAR was intercepted.
g. Nobel cites data from various authors, relating to un-irrigated crops of *Opuntia*.[71]

These four datasets are presented in Figure 7. Collating these into a single meta-data set carries some risks as it is not clear what conditions each trial or observation faced, though the implications from the literature are that the data represent unfertilised rain-fed crops. Nonetheless, the data present a coherent picture of productivity being water-limited, with a more or less linear response to water availability. The importance of this is that it demonstrates the resilience of these plants to drought – crop failure is not the issue – as there is only a proportionate decrease in productivity. Note also that these data represent observations where it is likely that field plantings were designed for manual rather than mechanised harvesting.

Mechanised harvesting could well increase yields per hectare substantially by reducing the space needed for harvesting access, thus allowing greater planting densities.

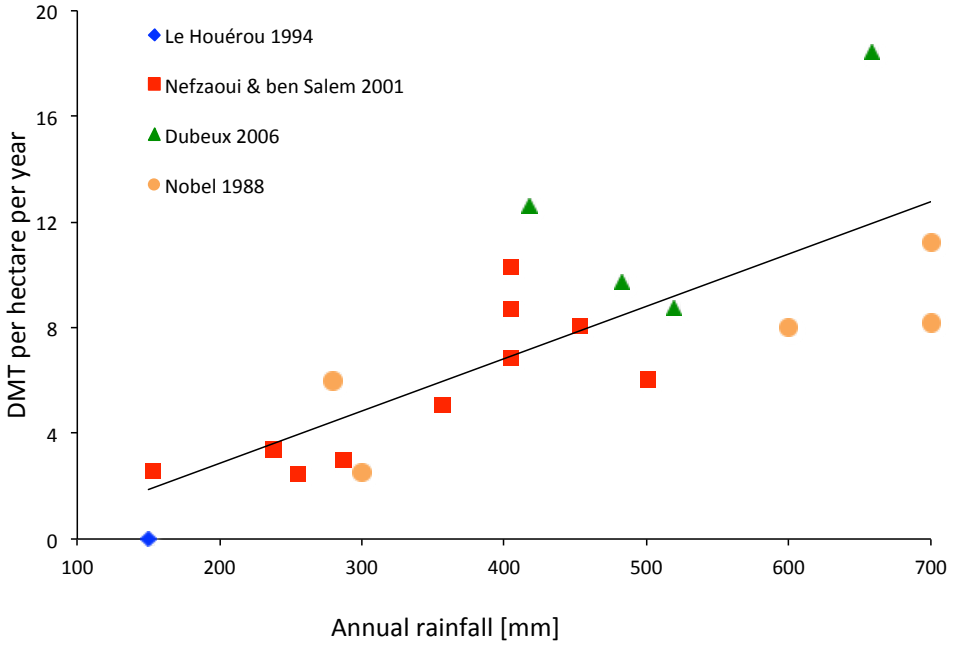


Figure 7 – Growth of *Opuntia ficus-indica* as a function of rainfall.

de Cortázar and Nobel used the Environmental Productivity Index (EPI) to forecast growth potential of *O. ficus-indica* globally.[72] This indicated 10-year average productivity of >10 dry matter tonnes ha^{-1} yr^{-1} over 40% of the land area where minimum temperatures were greater than −10°C. Yields of 12–18 tonnes ha^{-1} yr^{-1} were typical for Africa outside the Sahara, with up to 24 tonnes ha^{-1} yr^{-1} in western South America.

In contrast to *Opuntia* species, *Euphorbia tirucalli* is not a palatable fodder crop, and so there is much less literature covering its growth and potential yield. However, there is some literature describing its potential as an energy crop. As noted earlier, Calvin highlighted the potential of *E. tirucalli*, though he had it in mind as a source of oil rather than biogas.[53–55] One possibility that should be considered is that it could be used as both a source of liquid combustibles and biogas.

In the early 1980s, Leakey carried out trials of *E. tirucalli* in a semi-arid area of Kenya. These results, which were briefly reported by deClerck and Smets, indicated that the crop was appropriate but it did not address issues of the economics or optimise the agronomy.[69] They

indicated that the yield could be of the order of 16–20 tonnes yr^{-1} of dry matter for plantations at densities greater than 80 000 plants per hectare.

Reliable estimates of yield as a function of rainfall are scarcer for *E. tirucalli* than for *Opuntia* spp. The only searchable data based upon field trials seem to come from the same Leakey trial quoted by Duke – who cites ca. 20 inches (500 mm) of rainfall.[67] Shao and Chu quote a range of 250–1000 mm, though no yield data are included.[73] Loke et al. quote 1500 mm as the ideal rainfall, though this is clearly not relevant to the semi-arid regions for which the plant is being considered.[34]

In an unpublished trial at Mutumayu in Laikipia, Kenya, Gasston et al. of Live Energies GmbH established test plots of *E. tirucalli* and *O. ficus-indica* in June 2012.[74] The plots were un-irrigated, and the area receives 500–600 mm rainfall annually. Growth data for different planting densities from this trial, quoted as fresh tonnes, are shown in Table 5. Taking 10% dry organic matter as an estimate, based upon the average for *O. ficus-indica* calculated previously, suggests biomass growth rates in the region of 40 tonnes dry matter ha^{-1} yr^{-1}. Whilst there is not sufficient data here to establish reliable quantitative results over time, this illustrates clearly the significant advantage of high planting densities.

Table 5 - Experimental biomass yields from a growth trial in Mutumayu[74]

Plants per ha	Mass per plant (kg)	Approx. fresh biomass (tonne ha^{-1})
Opuntia, Sampled 17 months after planting		
3,333	11.0	37
8,000	10.5	84
20,000	33.0	660
Euphorbia, Sampled 12 months after planting		
66,667	1.5	100
133,333	1.0	133
266,667	1.5	400

These data are comparable to experimental yields quoted by Nobel of 43 tonnes ha^{-1} yr^{-1} and reinforce the observation that CAM plants can be as productive as more conventional C3 and C4 crops under conditions of much lower water availability.[29]

Gas yields

There is limited published literature about the biogas yields from CAM plants in AD. For crops that are digestible by ruminants, given that the basic processes of AD and ruminant digestion are closely related, and because biomass creates more or less the same amount of methane per unit of digestible material, knowing how much organic matter is present in the feedstock provides a proxy for relative productivity. *Opuntia* is frequently used for forage in N.E. Brazil, where something like 400 000 ha are in cultivation, and in other areas of the world.[42,57,58,61,66,75,76] Gasston's unpublished trial shows rapid digestion and good gas yields for *O. ficus-indica*.[74] Gas yields of around 325 l kg-1 (Figure 8) are consistent with gas yields from other forage biomass types. For comparison, maize grown as an energy crop produces around 300–375 litres CH4 per kg organic dry matter.[77]

E. tirucalli is not used as forage, and so biomass alone cannot be used as a guide to gas yield. Extracts are reportedly toxic to some mammals and birds and an antibiotic, which creates some concern for its ability to be digested in an AD plant that relies on a thriving micro-biome.[67] Nonetheless, three groups have tested its biogas potential and not reported any toxicity issues.[37,40,68] Further long-term testing is needed to confirm this conclusion.

Hastilestari et al. tested a range of different provenances of *E. tirucalli* for methane yield under AD (Table 6).[37] The four-fold variability in these data is uncharacteristic of gas yields from other crops, and suggests either a methodological flaw, or perhaps the influence of inhibition by toxins. However, the high yield in the Kenyan trial suggests that careful selection of cultivars and optimisation of the AD process to match the feedstock should allow yields of at least this magnitude to be achievable.

Digestibility tests on samples from the Mutumayu trials were performed at the University of Hohenheim.[74] Unpublished results from this work are shown in Figure 8. The *E. tirucalli* data shows somewhat lower methane yields than either *O. ficus-indica* or the tests by Hastilestari.[37]

Table 6 – Specific methane production from *Euphorbia tirucalli* grown in different locations, adapted from Hastilestari et al. [37]

	CH_4 yield (l/dry kg)
Togo	79
USA	161
Morocco	187
Senegal	238
Rwanda	214
Kenya	318

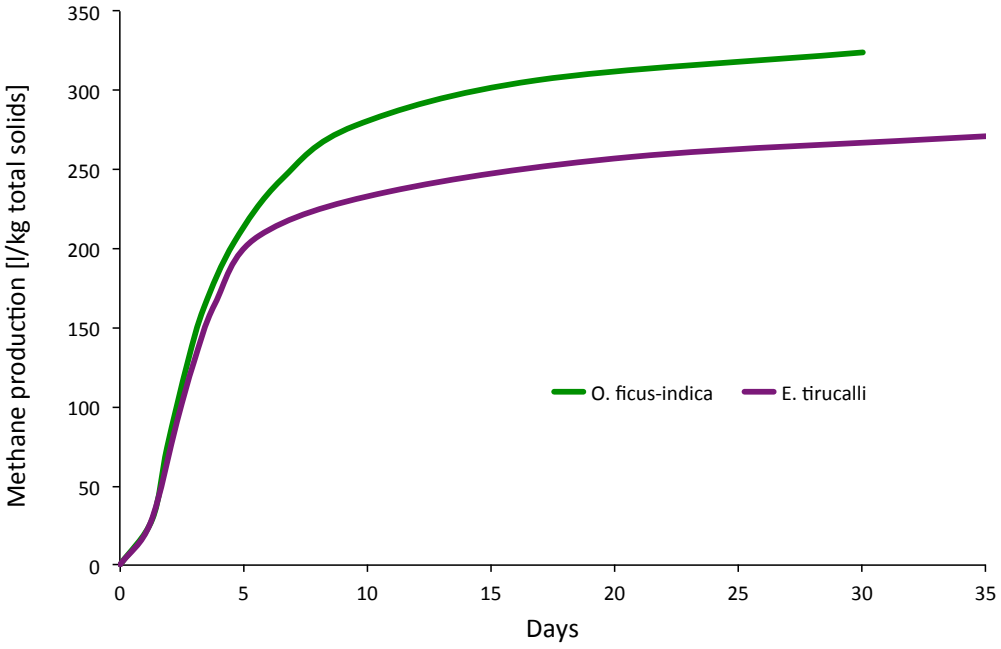


Figure 8 - Digestion rate of *Opuntia ficus-indica* and *Euphorbia tirucalli* grown in Laikipia, Kenya.[74]

GLOBAL LAND AVAILABILITY AND GENERATION POTENTIAL

Estimates of the global availability of semi-arid land where CAM crops could be grown without significant competition from agriculture are variable.

The Food and Agricultural Organisation of the United Nations (FAO) estimate 12.2% (1.8 billion ha) of the world's land area to be semi-arid.[78] They define this as 300–800 mm rainfall if the rains are summer rains, and 200–500 mm rainfall if winter rains. Davis et al. cite a figure of 18% (2.7 billion ha).[32] Kline et al. highlight FAO estimates of idle crop lands ranging from 0.52 to 4.9

billion hectares.[79] Adding together the estimated semi-arid land area as defined by FAO and the lowest of the estimates for idle crop lands, as representing potential sub-humid lands available, would suggest a global estimate of 2.3 billion hectares of land possibly available for CAM energy crops, but a range anywhere between 2 and 5 billion hectares is plausible (cf. Owen 2015).[80] Alexandratos and Bruinsma, using data from the FAO Global Agro-ecological Zones Study, estimated 2.9 billion ha of land to be marginal, or very marginal, for rain-fed agriculture, of which 220 million hectares is in use for rain-fed crops.[81] For the purposes of this analysis a nominal figure of 2.5 billion hectares is assumed.

Of this 2.5 billion ha, much will be unusable for a range of reasons. Terrain and accessibility to power evacuation infrastructure are two key factors. A third is the need to preserve ecosystems.

Clearly, in an ideal world, there would be no loss of ecosystems, but that would leave no room for human activity and welfare. The UN Convention on Biological Diversity seeks to address this through targets for land to be set aside for conservation. The Convention established the Aichi Biodiversity Targets which are to be translated into national action plans for the 193 signatory countries to the convention.[82] Target 11 seeks to ensure that at least 17% of land area should be set aside for conservation by 2020. Whilst this establishes a minimum area to be set aside it does not determine a maximum, or optimum to be set aside, or seek to influence further the inevitable social debate about the proper amount that should be used for agricultural or industrial purposes. It is also important to recognise in this debate that there is very little truly pristine wilderness left.[83] Kareiva et al. prefer to frame the conservation debate in terms of "*a discussion of what trade-offs we are willing to accept as a result of the domestication of nature*". Furthermore, it is likely that most of the land that would be most suitable for CAM energy crops is already grazed, or even over-grazed, by pastoralism and ranching.

One approach to this question is to ask how much land would be needed for energy crops to make a material contribution to global greenhouse gas emissions reduction, and then consider whether this was an acceptable trade-off. Table 7 sets out a synopsis of the calculations of energy generation potential of CAM plants. It considers a range of forecasts for growth rates, using the Laikipia gas yields from Figure 8. In the interests of conservatism, it does not include the higher gas yields from Hastilestari for the Kenyan provenance of *E. tirucalli*.[37]

Table 7 - Energy generation potential from AD using CAM crops as a feedstock

Electricity yields	*Opuntia* (low)	*Opuntia* (likely)	*Opuntia* (Mutumayu)	*E. tirucalli* (Leakey)	*E. tirucalli* (Mutumayu)
Dry tonnes yr^{-1} ha^{-1}	10	12	40	20	40
Gas yield (CH_4 l kg^{-1})	325	325	325	260	260
Energy in biomethane (GJ dmt^{-1})	11.59	11.59	11.59	9.28	9.28
Efficiency of AD process	64%	64%	64%	52%	52%
Electricity from biomass (MWh dmt^{-1})	1.33	1.33	1.33	1.06	1.06
Overall efficiency biomass to power	27%	27%	27%	21%	21%
Gross thermal power (W m^{-2})	0.57	0.68	2.28	1.14	2.28
Net electrical power (W m^{-2})	0.15	0.18	0.61	0.24	0.49
Total PWh at 100% of land use	33.3	39.9	133.0	53.2	106.4
Ha needed to produce 5 PWh yr^{-1}	**3.8E+08**	**3.1E+08**	**9.4E+07**	**2.3E+08**	**1.2E+08**
Proportion of available land to produce 5 PWh yr^{-1}	**15%**	**13%**	**4%**	**9%**	**5%**

A recent analysis of the availability of land for energy crops was carried for eight sub-Saharan African countries.[84] The analysis considered all agriculturally suitable semi-arid and arid land, and excluded all land with competing uses, including pastoral land and land where agriculture existed but was either marginal or could be enhanced by energy crops. Nonetheless, it demonstrated that approximately 10% of the total semi-arid and arid land was available for energy crops.

GLOBAL SIGNIFICANCE OF CAM CROPS

Coal is the world's biggest source of electricity, with approximately 9 PWh generated in 2011. The second biggest source of power was gas, at almost 5 PWh[4]. To achieve 5 PWh from CAM plants would require somewhere between 4% and 15% of the 2.5 billion hectares of potentially available semi-arid land, depending on the yield and gas production assumptions used.

The potential exists to be somewhere at the lower end of this range of land requirement if the Leakey trial data on growth rates are confirmed, and the Hastilestari data on gas yields are supportable with long-term testing.[37] Furthermore, there may be considerable opportunities to

improve both growth and gas yield as understanding of the agronomy and variability of the estimated 16 000 species of CAM plants improves.

Thus, there are reasonable grounds for optimism that a globally significant reduction in greenhouse gas emissions, and with that a substantial reduction in the risk of catastrophic climate change, might be achieved with only a modest loss of semi-arid habitat. The research presented in Chapter 4 suggests that this may be possible with no loss of food production. Food production may even be increased as a consequence.

2.3 The Complementary Resource Base - Solar

Neither the grid nor the climate system care if renewable electricity comes from AD or solar or any other source. The critical factor is that the source should be able to be integrated into the grid and provide power when needed and not just when nature delivers it. In this respect, AD has an important characteristic that distinguishes it from many renewable energy technologies. AD produces gas which can be stored cheaply in a plastic bag for periods of the order of a day. The gas can be used as and when needed in an engine, though this means a small extra cost for generator capacity to deliver peaks rather than average power. This means that it can be used to meet grid demands without the addition of batteries. Whilst this is an important component of the value of AD, it doesn't add specifically to the resource. However using the gas as a low cost efficient energy store does mean that it also be used to back up solar PV, thus increasing the total usable renewable energy resource available to a grid.

Solar PV is now cost effective as a source of power – particularly in the sunny regions where semi-arid adapted CAM crops will grow. A recent study for Tropical Power, (unpublished), showed expected solar yields from a 12MW DC solar farm at Naivasha in Central Kenya. Based on current costs (2014) and modelled performance the solar park will generate power whose cost varies as a function of the cost of capital as shown in Figure 9. The cost model for this graph is shown in "Gorge Solar from 1st Solar.xls" on the Supplementary Information disk.

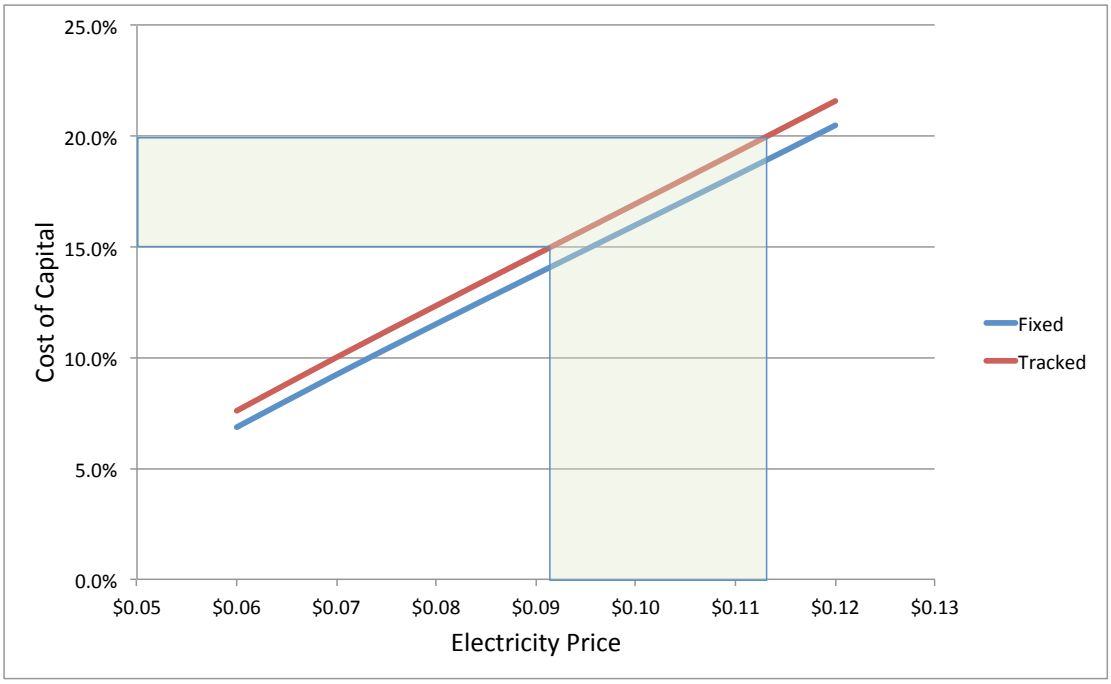


Figure 9 - Electricity Price as f(Cost of Capital) for central Kenya based on economic analysis by Tropical Power Ltd.

This clearly demonstrates that for a costs of capital of around 15% solar PV is now cost competitive with new coal fired power in some areas at least. As solar costs fall further these economics can be expected to improve, and make solar PV a compelling investment provided the grid can accommodate both the cyclical nature of the power and its extreme variability.

In principle, therefore, solar PV could be used to meet midday demands, and biogas used to fill in when the sun isn't shining. This will add a small amount of cost compared to an AD baseload plant because the electrical power output will be increased – so requiring a bigger engine and generator, but increase the amount of renewable energy that can be provided on either a baseload or load-following basis. In effect this stretches the AD resource base by making use of the ease of storing biogas.

One way to measure the value of the storability of the AD gas is to determine how much solar PV could be added to the grid for every MW of AD, and how much additional despatchable energy could be obtained as a result. To assess the scale of potential increase in despatchable electricity requires detailed models from each solar installation area, and a knowledge of local and regional grid characteristics. The following paragraphs use a mixture of synthetic data and real grid information for Kenya to generate some rough estimates.

In order to generate an upper limit estimate of potential daily solar power a simplified solar model was built using the approximation developed by Hottel.[85] The model is entitled "Solar generic model compared to Kenya grid.xls" on the Supplementary Information disk.

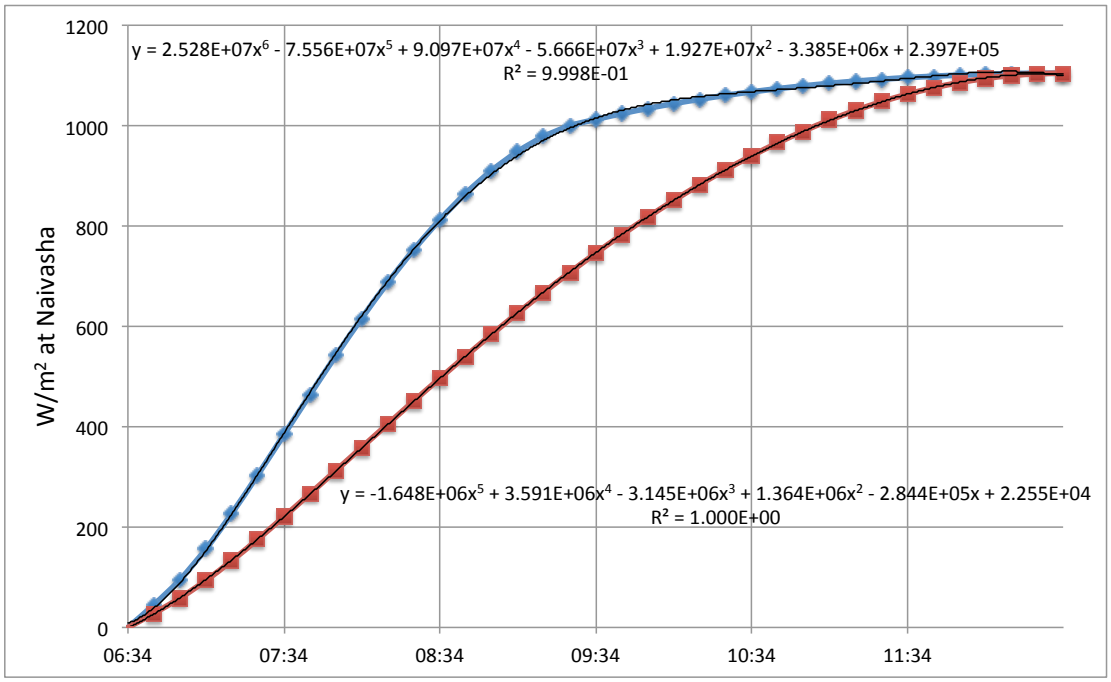


Figure 10 - Theoretical solar output from tracked and untracked systems, based on calculations developed by Hottel, and using a site at Naivasha, Kenya owned by Tropical Power Ltd.

Figure 10 shows the calculated radiation on a fixed horizontal plane, and a single axis tracked plane with 45° backtracking over half a day. These curves are for Naivasha, in the Rift Valley in Kenya, at an altitude of 1,900m. This is an area in which rain fed agriculture is very seasonal and variable, and in which both *E. tirucalli* and various forms of *Opuntia* are widespread. Note that the tracked curve shows a much flatter peak power output – which is much better suited to matching the requirements of a national grid.

Figure 11 maps this theoretical solar yield onto a typical day of the Kenyan National Grid, with the midday solar peak matched to midday demand. Total daily demand on the grid is approximately 18GWh, and the total solar output is 7GWh, or 39%. Thus for an AD plant in a sunny area with no clouds, the total output of electricity could be increased almost 63% [i.e. 39/(100-39)] without compromising the ability of the combined facility to meet the national load profile. This sets an approximate upper limit on the potential gains from hybridising solar and AD.

In the real world, however, clouds exist, and the atmosphere is dusty. As a result the actual solar energy harvested can be considerably less than the theoretical quantity. The widely used SolarGIS [86] system was used to calculate a likely in-plane radiation yield for the same site with single axis tracked crystalline silicon panels. This gave an annual generation of 2663 kWh/kWp, or 7.30 kWh/kWp per day. Thus 800MW of solar PV would deliver 5.86GWh/day. Assuming that Naivasha is typical of the semi-arid areas CAM plants might grow, a solar AD hybrid plant designed to output power in line with grid needs would produce 32.5% of its output from solar PV (5.86/18) and 67.5% from AD. Considered from the perspective of the AD resource, this would be effectively increased by 48% (32.5/(100-32.5). Detailed calculations supporting this are presented in the model "Solar generic model compared to Kenya grid.xls" on the Supplementary Information disk.

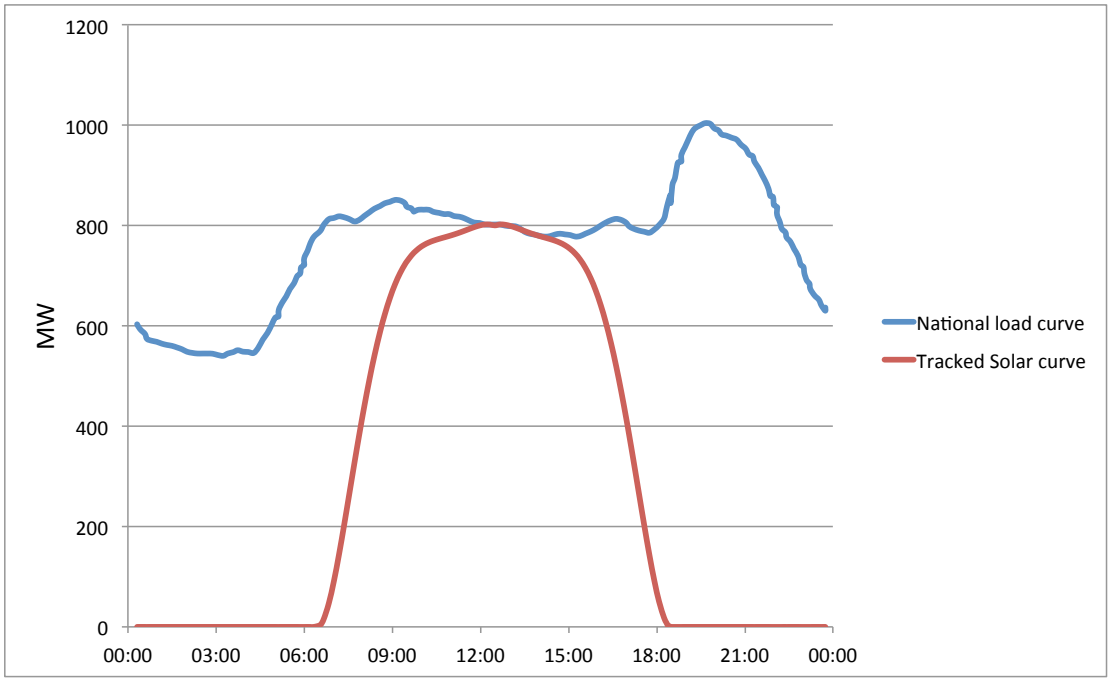


Figure 11 - Kenyan grid typical daily profile compared to forecast tracked solar output.

Some practical and economic considerations will affect this estimate. These include:

1. The problems of controlling a biogas engine to manage output to match very short term variations in solar output. These can take place over seconds rather than minutes. This is a soluble technical issue but as yet there are no biogas solutions developed – largely because of a lack of demand. This may reduce slightly the actual contribution from solar as a proportion of the AD output.

2. As the cost of solar PV falls even further, it will become economic to build oversized solar fields to match an AD plant, and spill any surplus electricity at midday. An oversized plant will only spill power when the sun is at its maximum – at all other times of day or levels of cloudiness it will simply contribute more to the hybrid plant. Inspection of Figure 10 shows that a 10% increase in solar output would result in substantially less than 10% of power being spilled, even in the absence of any clouds or dust. This will allow the contribution from solar to be increased.
3. This analysis presumes co-location of the solar and the AD plant. The benefit of this is that power evacuation infrastructure for solar PV can be an expensive part of the cost mix as its peak capacity (which determines cost) is used for only a small part of the 24 hour day. However in many instances this may be unnecessary. This would allow PV to be installed in the sunnier areas of a country and the AD in less sunny and wetter areas – though the limits on this will be set by the capabilities of the grid.
4. The areas which are closer to the higher estimate will tend to have lower rainfall and thus lower CAM plant potential – whereas the areas close to or below the Naivasha figure will tend to have greater agricultural potential. Thus lower AD production areas will generate more usable solar PV, up to the 63% maximum increase in energy calculated above.

Thus it would seem reasonable to assume that despatchable power from AD/solar hybrids can be increased by in excess of 40% compared to the power output of the AD plant alone at no great cost penalty.

Chapter 3 THE ECONOMICS OF AD

In the introductory chapter a target cost for AD was established at around $100/MWh in order to compete with coal. The aim of this chapter is to determine how much of a cost reduction is needed for AD to achieve this, given the likely cost of various feedstocks.

The chapter examines the cost of AD currently, and how that cost is made up. The introduction considers broad cost estimates from Germany, where the industry is well established, to provide an initial baseline estimate of the separate costs of feedstock and plant in Europe. Although Europe is where most commercial AD plants have been built, it doesn't provide an ideal reference cost because of the predominance of energy crops in the feedstock, which are grown on land suitable for food production, and the need to accommodate winter conditions. Winters prevent the distribution of liquid digestate on the land – so creating additional storage costs. A better example, although at this stage data is only available for one example, is Kenya, where crops can be grown under irrigation and thus are available all year, and digestate can be removed to the land all year also. Thus Kenyan costs represent the true cost of AD rather than reflecting ancillary elements.

The next part of the chapter considers the cost of feedstock, in order to determine the key variables, and the economic differences between feeding with crop waste or CAM plants. A further section assesses the cost of AD on a free feedstock basis. The final section combines these results to carry out a sensitivity analysis looking at the economics of potential generation from AD as a function of a number of variables.

The economic data and modelling used in this chapter is largely based on the known plant costs and performance of a specific 1.8 MW AD plant built in Naivasha, Kenya, by Tropical Power, and for which the author is both Chairman and Chief Technical Officer. This provides a realistic platform against which to extrapolate the economics of a range of situations and crop possibilities.

3.1 CURRENT AD PLANT COSTS

THE CURRENT COST OF AD IN GERMANY

The current cost of electricity from AD varies significantly from place to place and feedstock to feedstock. However some indications can be found from the levels of economic incentive available throughout the world.

The largest number of biogas plants in the world, by several orders of magnitude, are domestic biogas plants for cooking gas. Nepal, India and China have, between them, perhaps 10 million plants [87]. Based on data from Nepal, each plant saves around 2 tonnes of wood fuel per year. Assuming an energy content of around 12GJ/tonne of fresh wood, each plant has an energy output of around 6MWh/year. Thus using these data, given that the gas is produced on a continuous basis even though it is used intermittently, the typical plant output appears to be around 680W. This however overstates the likely output, because wood fires are notoriously inefficient. A generous estimate might be 350 W of gas – equivalent in electrical terms to around 100W(e) at 35% conversion efficiency. Thus total output comes to around 1GW(e). For these plants the economic incentive comes from the replacement of either expensive wood, or bottled gas. Many are also subsidised by government programs, so the provide little evidence for the economics of AD.

Germany is believed to have the largest installed capacity of AD plants generating electricity globally, with around 10,000 commercial plants, generating 6GW, giving an average electrical output of 600kW(e). In Germany, generous feed-in tariffs stimulated rapid growth of AD plants, but the recent reductions in the tariff have resulted in a significant slowdown of growth, suggesting that the tariffs are now more or less insufficient to be economically viable.[88] Tariffs, excluding special payments for CHP and manure use, were as in Table 8.[89]

Table 8 - German Biogas Tariffs

Size	2009	2012
Up to 150 kW	$0.249	$0.191
Up to 500 kW	$0.216	$0.164
Up to 5 MW	$0.163	$0.147
Up to 20 MW	$0.104	$0.080

This suggests that the economic cost of AD in Germany is between $0.16 and $0.22 for smaller plants up to 500kW, and between $0.15 and $0.16 for plants between 500kW and 5MW. Figure 12 [90] shows that these two sizes cover the majority of plants in use of any significant size .

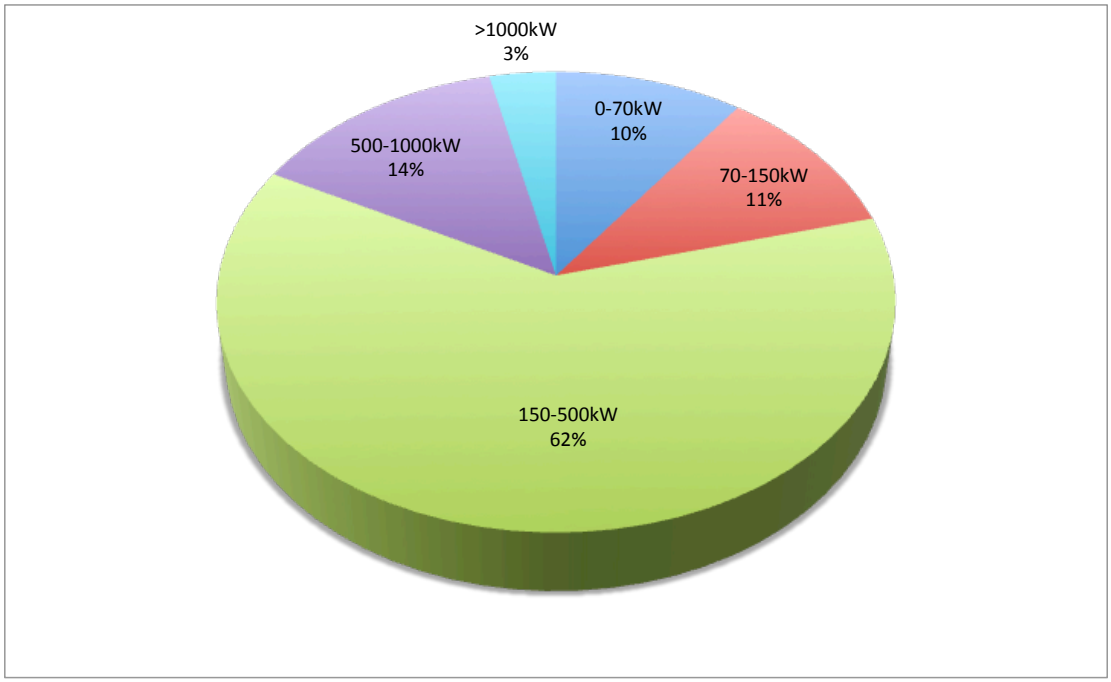


Figure 12 - Distribution of sizes of German AD plants

The principal feedstock for many plants in Germany is forage maize. A widely used estimate for the breakeven cost of maize into AD plants is around $40/fresh tonne [91] with a range between $27 and $71 per tonne.[89] Using unpublished data from Snow Leopard Projects, a German AD developer, a fresh tonne of forage maize silage will generate approximately 366kWh of electricity from a single stage AD plant. This translates into a cost of around $0.109 per kWh(e) for $40/tonne of feedstock. Back-calculating from this can offer a sense of the cost of owning, operating and financing an AD plant in Germany (Table 9).

Table 9 - German AD Plant Costs

Size	2009 tariff	Feedstock cost	Cost of plant and operation
150–500kW	$0.216	$0.109	$0.107
500–5,000kW	$0.163	$0.109	$0.054

Personal experience is that German AD plants operate more or less automatically – with no management other than daily feeding by the farmer, so no significant allowance needs to be made for overheads.

Several features stand out in this rather approximate analysis. Firstly, feedstock from purpose grown crops on good agricultural land is currently too expensive to allow AD to compete with coal. Secondly, the implied cost of a smaller plant is perhaps double that of a larger plant.

An alternative view might simply be that this is a more attractive tariff, which also accounts for the fact that there are over 4 times as many of the 150-500kW plants than there are of the 500-5,000kW plants larger plants. There are, however, several other possible explanations for this.

- 500kW may simply reflect the size of German farms – which highlights the importance of developing an AD system that is economic at smaller sizes.
- Over 500kW it may only be possible to make AD work in areas of lower feedstock costs.

There are clearly many more uncertainties surrounding the true economics of German AD, but the data presented here serve to give an understanding of the real world economics as they exist today in Europe with purpose grown energy crops.

The Cost of Owning and Operating an AD Plant in Kenya

Germany is a country with a cold winter and a single annual harvest. Feedstock therefore has to be stored for many months. Cold weather also means that the waste liquid from AD plants has to be stored over winter to be spread on the land in the summer – adding to costs.

An alternative model is one where the crop is available throughout the year. This can be either because of continuous harvest under irrigated conditions, or the use of crops such as CAM plants

(discussed later) which can be harvested continuously even under variable seasonal rainfall conditions.

In the interests of developing a realistic model of the costs and investments needed for an AD plant, without the extraneous factors of crop storage and liquid digestate disposal the economic modelling has been based on cost data from an actual plant built in Kenya. The plant has been built to run on vegetable waste from the Gorge Farm and some adjacent farms. This is mostly (80%) maize straw from commercial baby-corn production, with the balance from broccoli, beans, and various minor additional components. The plant design has tank sizes as in Table 10. The nominal capacity of the plant is 1.8MW(e), giving a power density of 0.23kW/m^3.

Table 10 - Gorge AD Tank Sizes

Tank Type	Height	ID	Number	Total Volume
Hydrolysis	8.0 m	11.0 m	2	1,521 m3
Digester	8.0 m	30.0 m	1	5,655 m3
Recyclate	8.0 m	11.0 m	1	760 m3
TOTAL				**7,936 m3**

The total cost of plant construction was as detailed in Table 11.

Table 11 – Kenya AD plant costs

AD Tanks and Civil works total	$4,237,159
Gas Clean-up Train	$304,904
Connection	$182,929
Engines	$1,729,549
TOTAL	**$6,454,541**

These costs are shown graphically in Figure 13. In addition $430,000 of equipment was needed for harvesting and haulage operations but as these are independent of the AD plant and only dependent on the crop and farm design they are not included.

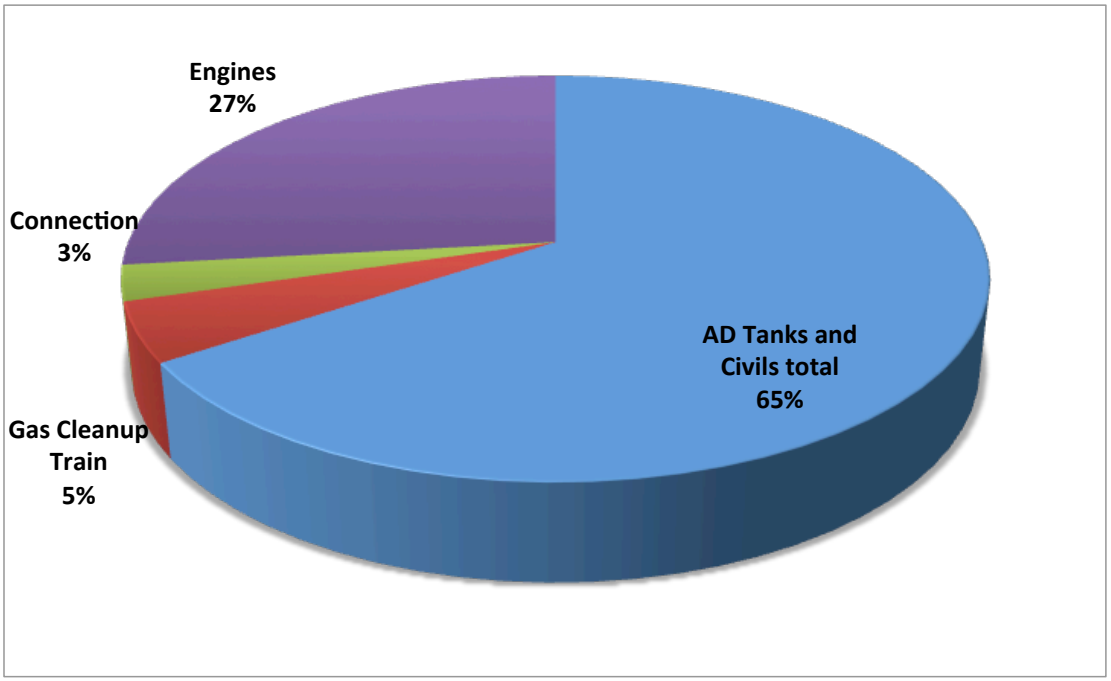


Figure 13 - Gorge AD Cost breakdown, derived from actual data for the construction of a 1.8MW AD plant in Naivasha.

"Ag and CAM economics.xlsx" on the Supplementary Information disk is the cash flow model used for this analysis. It is based on actual equipment and costs for Gorge Farm. It includes costs for building and operating the plant and the harvesting equipment, but not for the feedstock itself, which in this case is agricultural waste.

The cash flow analysis for this plant shows a project IRR (i.e. unfinanced) of around 13%, before tax and management charges, and working capital requirements. (*In this case the income is supplemented by sales of heat, and sales of electricity at retail prices, which are higher than the feed in tariff. These are not included in this 'bare bones' project valuation – in order to show the economics of AD in isolation*).

3.2 Resource Economics

In order to understand the cost reductions that may be needed to AD plants in order for them to deliver significant power at a cost competitive with coal it is important to understand the feedstock costs – because these are largely fixed by other parameters and difficult to manipulate.

Municipal Solid Waste

Municipal Solid Waste (MSW) is difficult to cost because its collection is a matter of public health and welfare – so it is usually paid for by local government. However disposal of the waste is then an expense for the collector, and often, in Europe, brings with it a gate fee to the disposer. There will be some cost of sorting to remove inert materials, metals, and other non-organic matter, but this is largely self-funding through the value of the recovered materials. There is also possibly a cost to pasteurisation before digestion, but as an AD plant has ample waste heat this is not a major cost.. Thus MSW is, to all extents and purposes a free resource where it is collected. Unfortunately, in the global scale of resources, it is relatively limited. The amount collected could undoubtedly be increased by improving the economics of AD, but it is difficult to determine any sensible metrics to understand what cost reduction would achieve what outcome.

Agricultural Residues

There are three components to agricultural residues; the value if left in or on the soil, the opportunity cost where there are alternative uses, and the cost of collection,

There are several reasons why crop residues might be left on the soil. Key ones are the control of erosion, management of soil organic content and retention of macro- and micro-nutrients.

Erosion control is has been studied in the US in the context of removing corn residues for bioethanol production.[92] The study concluded that 40% of residues could be removed under conventional agriculture and up to 70% if erosion control was the sole objective. Some element of amelioration of this position can be achieved by returning the solid digestate to the soil, [93]. This, then, is not so much a cost or value, as a fundamental requirement which will vary site by site and limit the total resource available.

As regards the other elements of value, including macro- and micro-nutrients, all of these can be returned to the soil if needed by returning the AD liquid and solid digestates. The cost of doing this is relatively low compared to the cost of harvesting and hauling the initial feedstock – the liquids can be pumped, and the remaining undigested solids returned on the back-haul of tractors bringing in feedstock. Even if the material has to be hauled by a separate fleet, the tonnage is minimal.

Opportunity cost is difficult to generalise. In different parts of the world, different opportunities exist, ranging from nil where the crop residues are simply burnt in the field, through areas where it is used as a low grade cooking fuel to Denmark where straw is used for combined heat and power plants. However none of these values are very high, and all suffer from the need to process and transport the residues – sometimes considerable distances. All that can be safely said about opportunity costs is that they are generally low, and the higher the margin for electricity production the less the opportunity cost will constrain the availability of resource.

The cost that all farms will face in the supply of fuel for AD is the cost of harvesting the residues, chopping them to an appropriate size, and hauling them to the AD plant. Figure 14 illustrates the cost of harvesting and hauling for a range of input parameters which derive from actual operational conditions at Gorge Farm.

All the following graphs are from the model "Ag and CAM economics" on the Supplementary Information disk.

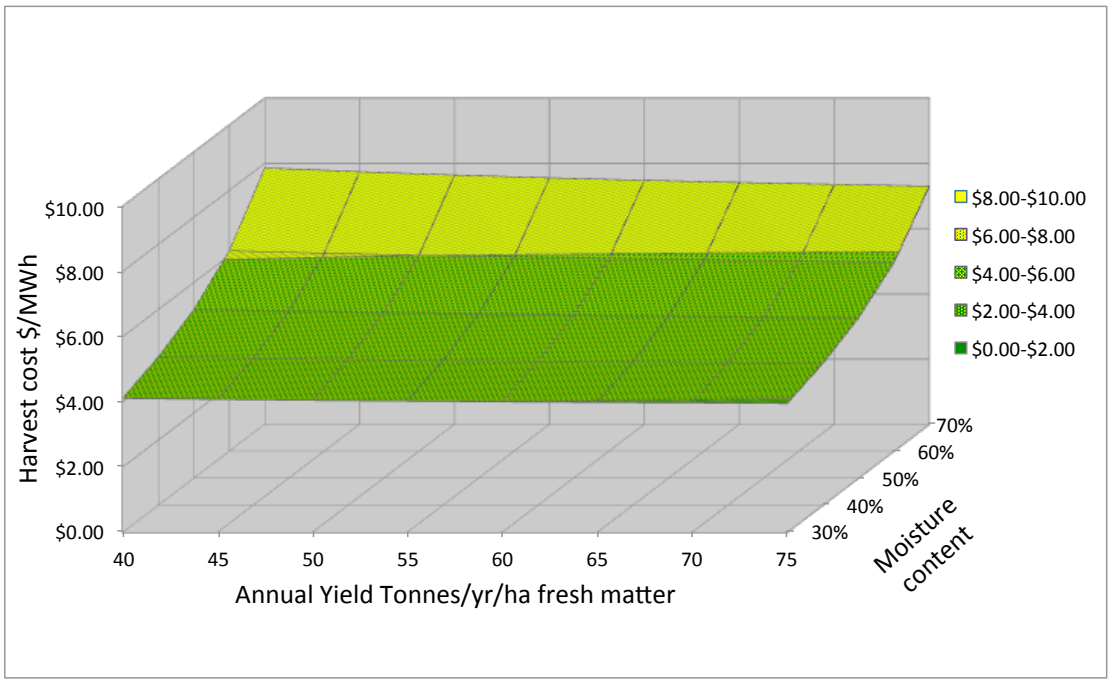


Figure 14 - Agricultural residue costs at Gorge Farm. Data modelled based on actual operating costs, including harvesting by forage harvester and hauling to the AD plant.

CAM Plants.

CAM plants are the potentially most important resource for AD. Unlike the other AD resources, the cost of planting and management has to be borne by the generation of power. The particular feature that distinguishes them, though, is their moisture content – which can exceed 90%.

Understanding the relationship between water content and cost is key to understanding the economics of CAM plants as an energy source. Moisture content varies by location, variety and across seasons as a function of rainfall – and a number of years of data will be needed to determine the specific data for any given site and crop type. This experimental work is currently in hand for three species; *Opuntia ficus-indica,* an as yet unidentified form of *Cylindropuntia* which is locally prolific, and *Euphorbia tirucalli.*

In the absence of hard data, an understanding of the potential range of economics can be derived from a simple sensitivity analysis such as is shown in Figure 15. Note that the scale of costs per MWh has increased by almost an order of magnitude compared to agricultural wastes, and that the sensitivity of costs to moisture content is particularly high above the 90% MC level typical of *Opuntias*. *E. tirucalli*, with MCs of 83% to 87%, would appear to be a more attractive crop.

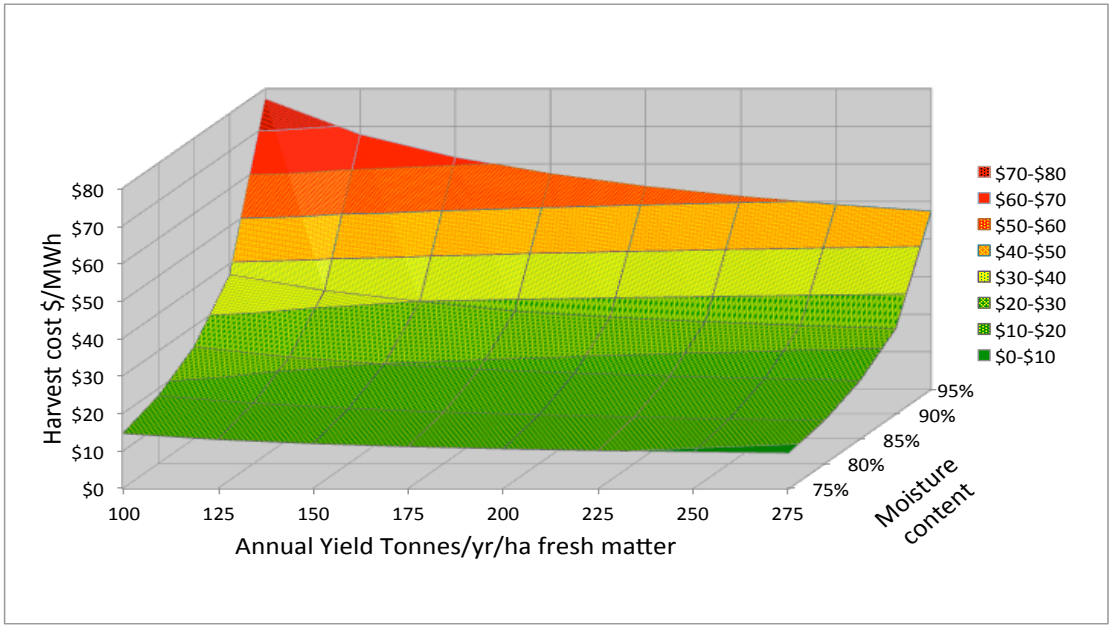


Figure 15 - CAM Plant modelled feedstock costs, based on known operating costs for Gorge Farm, but adjusted for the different yields and moisture contents of CAM plants. In reality dedicated large scale farms growing CAM crops would use rather different equipment, including conveyors or light railways – as are widely used in sisal farms – with a resulting lower cost than shown here.

3.3 DISCUSSION

It is clear from the German data presented earlier that the cost of the feedstock alone precludes reaching parity with coal for dedicated conventional energy crops – regardless of how much the cost of AD might be reduced. Nonetheless, the cost of the AD, at an implied $54/MWh for plants between 500 kW and 5 000 kW, suggests that in countries where there is a low cost fuel supply, AD can more or less compete with coal even today. These figures are, however, somewhat misleading because they also reflect the very low cost of capital in Germany – something that doesn't apply in the developing world. The "Ag and CAM economics.xlsx" model also shows that you need around a farm of around 200 – 250 ha to feed a 500kW plant, and the German tariff data suggests that below this size costs increase substantially. However most of the world's farms are nowhere near this size. Adamopoulos suggests that the average farm size in the poorest 20% of countries is 1.6 ha, and even in the 20% of richest countries it is only 54.1 ha.[94] Clearly very substantial cost reductions are needed to access these farmers – even if small groups were to band together to operate a single AD plant. Admittedly the value of power in these circumstances may be significantly more than the $0.10cts target of this work, but the scale is tiny compared to commercial AD.

The Gorge farm presents a somewhat different picture. This is a large commercial farm providing flowers and vegetable to the European supermarket trade. It is a sophisticated 'first world' operation embedded in a less developed country (LDC). The costs for electricity generated from the AD plant on this farm are interesting because they are 'clean'. Feedstock costs include only the marginal cost of harvesting and transporting the crop residues; harvesting is continuous so there is no need for crop storage; and digestate removal and distribution is possible continuously so there is no cost for liquid digestate storage. (In Europe and the US, winters preclude the spreading of nitrogen rich liquid digestate outside the main growing seasons to avoid excess nitrogen on the land finding its way into water tables and water courses).

The "Ag and CAM economics" model builds up the costs of harvesting and hauling the feedstock (used in Figure 14), and building and operating the 1.8 MW AD plant at Gorge Farm. The 13% IRR pre-tax and overheads would generally be considered a modestly attractive power project in

the developed (largely temperate) world. However the additional costs of crop and digestate storage that are imposed by winter conditions in the temperate zones means that, as a general rule, subsidies in some form or other are currently needed to support AD plants.

In LDCs, the Gorge farm 13% IRR is at the lower limit of acceptable rate of return for large fixed projects. Thus in the rather ideal circumstances that this farm offers, the plant is barely viable at $0.10cts/MWh.

Just as with the German temperate climate example, or the small scale farmer example, avoiding subsidy needs cost reduction. There is little or nothing that can be done to reduce feedstock costs from agricultural residues – so any cost reduction has to come from the capital cost. Using the Gorge financial model, a sensitivity analysis was prepared to assess the impact on rates of return to investors of a reduction in the two major components of capital cost – the tanks and civil works, and the engines (refer to Table 11 for details).

Figure 16 shows a plot of the rate of return of the Gorge Farm AD plant as a function of independent reductions in engine cost and civil construction cost. The analysis is based on a sales price of $100/MWh of electricity. A 40% reduction in civils costs, or a 60% reduction in engine costs (or some combination of the two) would lead to a welcome comfort factor in the investment decision, leading to a pre-tax project IRR of just over 20%.

The bulk of the AD opportunity is, however, neither in the small farms nor in the large farms such as Gorge, but in the CAM plants grown in semi-arid areas.

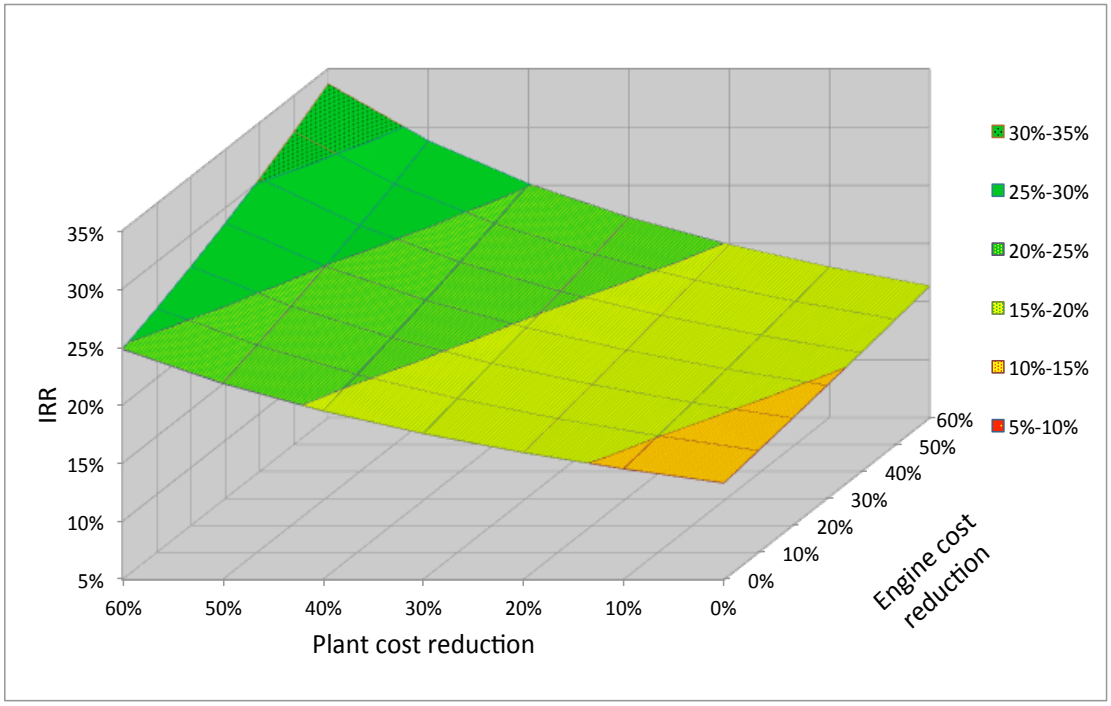


Figure 16 - AD cost sensitivity based on agricultural residues. The graph shows how IRR of the Naivasha plant would vary as a function of reduction in cost of the engines and the AD plant.

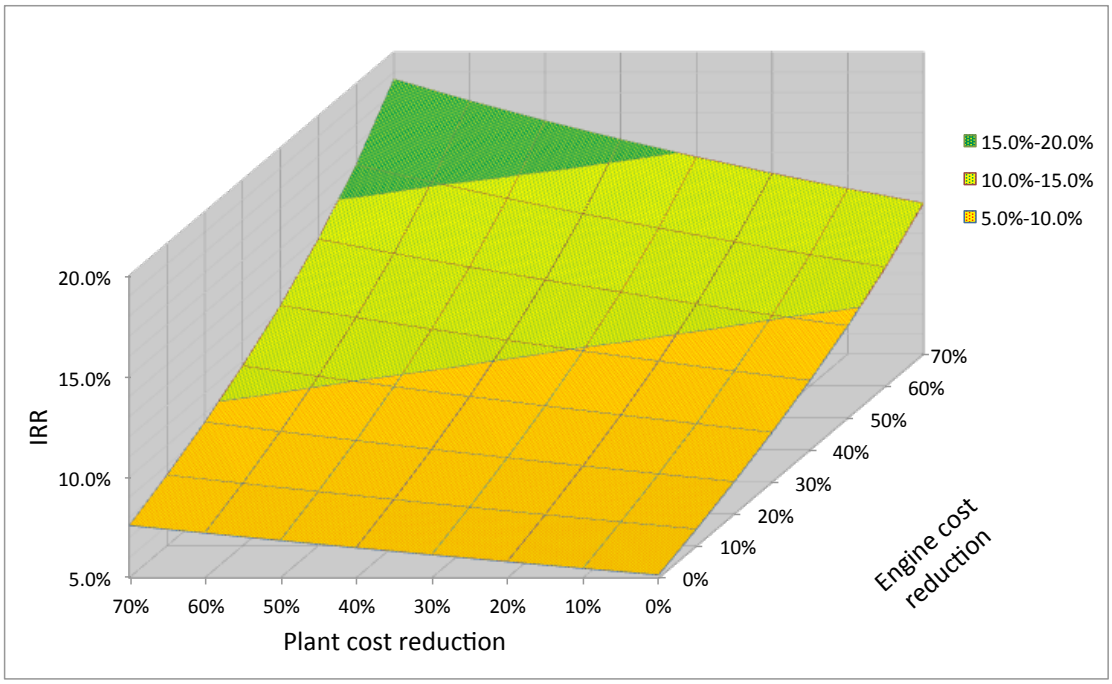


Figure 17 – AD Plant cost sensitivity based on CAM plants, once again taking the Gorge AD plant costs, but flexing them to match the yields and moisture contents of CAM plants.

Figure 17 repeats the analysis of Figure 16, but using the CAM plant harvesting costs as shown in Figure 15. A moisture content of 90% is assumed, with a fresh matter yield of 200 tonnes/ha/yr.

A different perspective on CAM plants can be gleaned by linking the savings from engine and civil works improvements – to give a single capital cost reduction percentage. Using the third axis then for moisture content gives the graph in Figure 18.

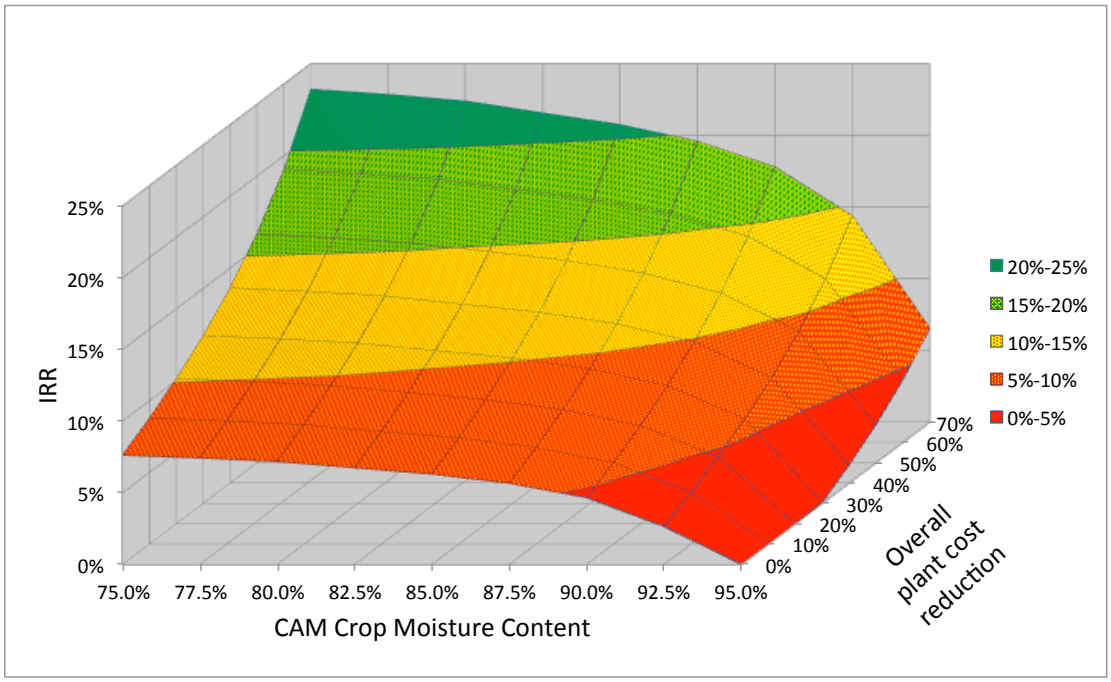


Figure 18 - IRR sensitivity to Moisture content of crop and cost reduction. In this graph the cost reductions in engines and AD plant have been combined to give an overall capital cost reduction. This allows moisture content to be added as an additional dimension.

This graph is a direct combination of Figure 15 and Figure 17. It highlights the extreme sensitivity to MC of the economics above about 90% moisture as well as the importance of cost reduction. A 85% MC and a 60% cost reduction across the board puts this in the same economic position, more or less, as Gorge Farm with no cost reduction.

The CAM model and the agricultural waste models here have used the same basic cost data to ensure comparability. However there are things that could be done to reduce the costs of CAM based AD. Increasing the scale of the harvesting equipment and of the AD plant; using conveyors for transport of feedstock; using the water collected for hydroponic or similar irrigation are three obvious ones. Undoubtedly there will be others that become apparent.

Chapter 4 COMPLEMENTARY AGRICULTURAL BENEFITS

AD produces two output streams, a solid indigestible compost-like digestate, and a nutrient rich liquid digestate. Both of these have potentially valuable additional uses.

4.1 SOLID DIGESTATE

The solid waste from AD is made up of the indigestible portions of the lignocellulose plus some bacterial matter. Lignin will generally be a significant component. A study of a mixed feedstock into a 1MW biogas digester with a 50 day retention time, over a period of 8 months, showed a ratio of solid digestate to solid ingestate of around 25%, with the lignin content increasing by a factor of over 400%.[95]

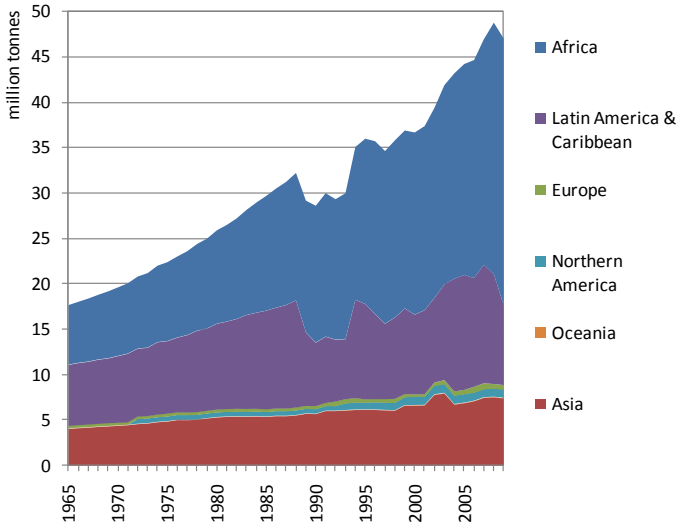


Figure 19 - Annual charcoal consumption worldwide.[96] (*Reproduced from the FAO.*)

Tong et al. report conversion efficiencies in corn stover of 84% for cellulose to biogas.[19] Adding around 10% additional lignin would leave 25% of the initial dry matter unprocessed, so confirming the previously cited data. This 25% figure may be somewhat high for many reactors where operator anecdote suggests 15% is more characteristic.

This solid digestate can, in principle, be briquetted and turned to charcoal. Many grasses have high levels of inorganic salts that contribute to high ash content and low ash melting points – both undesirable features of quality charcoal. Because this digestate will have had much of the soluble inorganic materials removed, it is likely to make better charcoal than the initial stover.

This solid material can be used as soil improver in exactly the same way as compost, but this may not be the best way to exploit its value. Charcoal is a key fuel for in developing countries and the growth of charcoal consumption has broadly mirrored increasing urbanisation and population (Figure 19). Around 2.5 Bn people use wood or charcoal as their primary cooking fuel. Charcoal production is also a major cause of environmental damage; not only does it result in massive deforestation [97], but also inefficient production methods cause significant greenhouse gas emissions [98,99]. AD solid digestate can be made into charcoal briquettes, and it is as charcoal that it is likely to create the highest revenue, in developing countries at least. Efficient charcoal manufacture from AD digestate could also be done with little or no net greenhouse gas emissions.

Assuming an efficient charcoal production system, 15% of the feedstock exiting as solid digestate would produce about 5%-10% of the feedstock mass as charcoal. This estimate is higher than might be expected from efficient wood pyrolysis, as a result of taking into account the very high lignin fraction. It appears that lignin has a much higher conversion ratio (over 70%), to charcoal than cellulose or hemicellulose [100]. More normal yields for efficient processes are 30%-35%, though some processes can yield over 60% [101]. Charcoal costs vary substantially but a typical price in Kenya and Tanzania would be around $150/tonne. At this price, and assuming a 33% conversion to charcoal, each tonne of feedstock would produce about 50kg of charcoal, worth of the order of $7.50.

Making an allowance of, say, $50/tonne of charcoal for the cost of pyrolysis and briquetting still leaves a net revenue of $5/ dry tonne of feedstock. Although this is only a modest 5% or so of the potential revenue it does make a useful contribution to the cost of supplying the feedstock, with the added benefit that feedstocks that produce less gas per tonne will produce more undigested material, and thus the more charcoal will be produced.

4.2 Liquid Digestate

Some of the water imported into an AD plant is used in the chemistry, some is lost by evaporation, but the bulk of the water input with the feedstock into an AD plant reports to the outlet of the plant as liquid digestate. This liquid digestate contains most of the soluble

inorganics that derived from the biomass, together with a proportion of the indigestible solids that form a colloid and thus is not separated out with the solid fraction of digestate [95].

For arable crops the water production may be minimal if the residues are harvested dry, but for CAM crops it can be substantial. Consider a crop that produces 10 dry tonnes per year per hectare at 90% moisture. Consumption by the AD process, calculated using the Buswell Equation [102] of about 13% will leave around 80 tonnes of nutrient rich water per hectare per year. This is equivalent to 8mm of rainfall. Although this is not enough to support agriculture over the entire crop growing area, it is enough to support drip irrigated market gardening or even hydroponics over a much smaller area. The absolutely regular nature of the water availability, the controllability of its delivery and its nutrient content means that highly productive agriculture should be possible anywhere where CAM plants can be grown.

A recent study in PNAS [103] quotes wheat under drip irrigation needing 1.4 ft (427 mm) of water, and giving yields of 115cwt/acre (14.5 metric tonnes/hectare). Thus 8mm of rainfall recovered from the feedstock area would allow about 2% (8/427) of the CAM crop land area to be used for highly productive agriculture. Assuming 10% of the available semi-arid land, i.e. 250M ha., is used to generate 5PWh as described in Table 7, a further 5M ha. of productive irrigated land would be added to the current global stock of approximately 100M ha of un-degraded irrigated land.[104]

The nutrient rich liquid waste could find other uses however, either before being used for irrigation, or instead of such use. Growing algae is one possibility, but perhaps even more promising is the growth of highly productive *Lemna* spp.

Lemna spp. are small, free floating, aquatic plants that are the subject of considerable interest as a source of protein.[105–108] They have prodigious growth rates, and can be 25%-40% protein, with the higher figures associated with the faster harvesting rates.[107] These protein concentrations are as high as soy beans, which command a market price of around US$1,000/tonne, and the material is highly palatable for both ruminants and fish[105,106,108–110].

The global market for fish is vast, and with pressures on natural stocks and a growing global population wanting to eat more, AD could be an important new and sustainable source of protein

for humans. Global production of *Tilapia* alone today is around 5 million tonnes/year. The Chinese market price is around $1.35/kg, valuing the global market at $7 Billion a year. [111].

4.3 Farm Income Security

Much of the world has enough rainfall on average for arable crops, but the variable timing of its arrival, and the variability from year to year, makes farming an insecure business. Having AD as a supplementary source of income using a combination of CAM crops and agricultural residues could conceivably increase the security of farm incomes. In good years the main feedstock could be food crop residues, leaving the CAM crops to accumulate biomass, and in bad years the accumulated CAM crops could be harvested to make up for the missing food crop residues.

In addition succulents such as the spineless varieties of *Opuntia* can be used as emergency fodder for livestock in times of drought, though they contain too much water to be ideal feedstocks when alternative grazing is available. Thus AD is an excellent potential complement to current farming practices and could add to farmer incomes and security.

Chapter 5 SUMMARY AND CONCLUSIONS FROM PART 1

Chapter 1 set out the need to deliver massive GHG emissions reductions in order to avoid the serious consequences of anthropogenic climate change. It highlighted the need to consider not only the cost of the technologies to do this, but also the ability to integrate them into a new energy system that is capable of generating renewable power when the customer needs it rather than when nature provides it. Wind and solar may be low cost technologies, but they are not despatchable. This limits their ability to provide the majority of a nation's energy requirements without radical improvements in both daily and seasonal electricity storage technologies.

The proposition is that for any renewable energy technology to make a significant contribution to global GHG reduction it needs to fulfil the following five requirements.

a. It must be the best way to use the resource available.
b. It must be able to compete on cost terms with fossil fuel alternatives.
c. It must be usable in existing energy systems at high levels of penetration.
d. It must be of a sufficient scale to have a material impact.
e. It must be sustainable at scale, and neither compete with food production, nor make a material impact on valuable ecosystems such as forests.

The chapter argues the case that AD is the best use of a major part of the world's biomass resource other than the woody biomass which is not amenable to easy digestion. Firstly, AD provides more useful energy per unit of feedstock than conversion to liquid fuels such as ethanol. Secondly, it proposes that the need for liquid fuels is a much lower priority for most of the world than electricity, and that this emphasis on electricity rather than liquid will grow over time – thus reinforcing the focus on AD and fulfilling criterion a) above.

The advantage of AD over other renewable forms of electricity generation (other than hydro-power from dams) is that it is to a great degree despatchable. Biogas can be stored overnight, to meet variable daily needs, and it is even possible to meet an element of seasonal variability by storing biomass for digestion at different times of year – albeit this has a cost implication. Thus AD also fulfils criterion c).

Chapter 2 considers both the existing and the potential the resource base. The existing resource base is comprised of two parts – municipal solid waste, and agricultural residues – with energy potential of 0.3 PWh and 1.7 PWh per annum respectively. These are material, but modest in the context of global generation from coal.

A much bigger potential resource could be found by using water efficient CAM plants on the massive under-exploited semi-arid or degraded areas of the world. It is difficult to find consistent data on either the usable semi-arid land area, or the energy potential of the crops. A number of studies point to areas of the order of 2.5 billion hectares. To generate 5PWh of electricity from this land would require between 4% and 15% of this area.

Figure 20 puts these data into context. The green bars show the potential of AAD. In addition the orange bars above the AAD bars show the solar contribution based on 40% addition to the total energy output of AAD as a result of hybridisation. This illustrates the material impact AD could have a on global GHG emissions if it were to achieve even a proportion of its full potential and demonstrates that AD can fulfil criterion d) above.

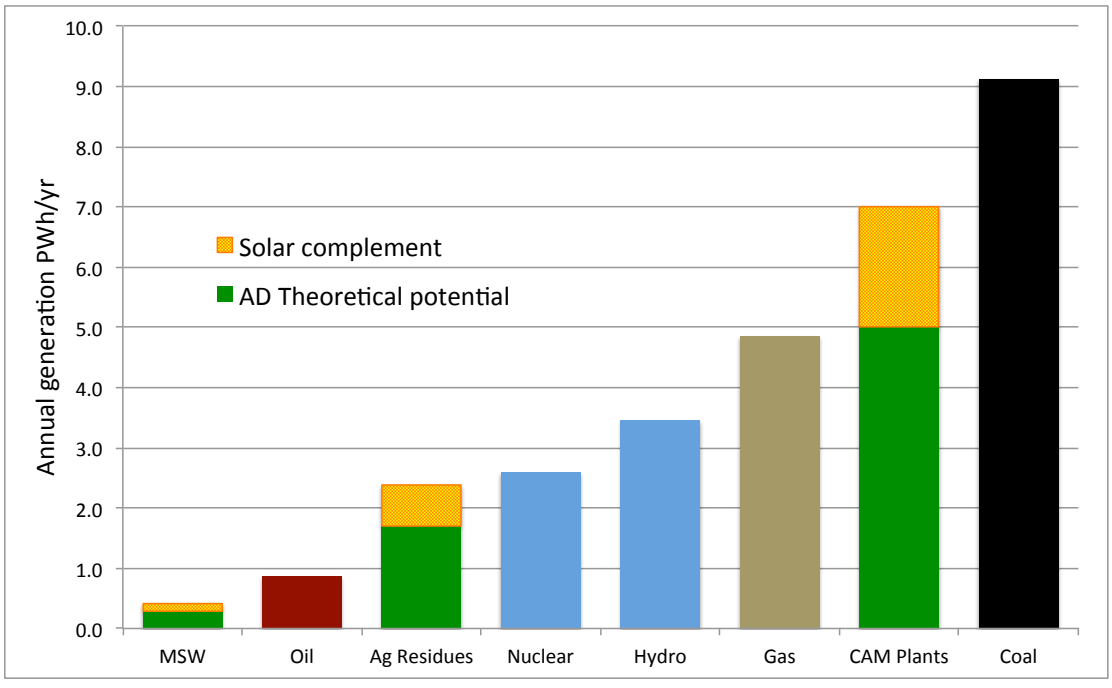


Figure 20 - AD Potential in the context of global electricity generation.[112]

To achieve this potential, the cost of AD has to be reduced. Chapter 3 demonstrates that AD is already almost cost effective at $0.10/kWh for a large plant (1.8MW) using agricultural waste

which has no cost other than the cost of harvesting and hauling. Smaller plants, however, are needed to capture a great proportion of the global agricultural waste resource, and the German data illustrates the extent to which this increases the specific cost (in $/kW). This limits the real world application of current AD technology to a sub-set potential sites unless some measure of cost reduction is achieved.

Although CAM plants offer the biggest potential resource for future AD, the cost of growing, harvesting and hauling them to an AD plant is a material part of the power cost, and precludes their economic use with current technology. For CAM plants to contribute to global power supplies the costs of growing and treating them need to be reduced significantly.

The most significant parameter for the plants themselves is their moisture content. Many CAM plants have a MC of around 90%. Inspection of the graphs showing the interaction between IRR and MC shows that 90% represents a key inflection point – much above this and CAM becomes so expensive to haul that no realistic level of cost reduction of AD plants is likely to enable them to be usable. Below 85%, on the other hand, MC becomes a second order issue. What is clear is that in the development of CAM plants as energy crops great attention needs to be paid to the MC, as even small reductions will make a significant impact on the economics.

The cost analysis in Chapter 3 is somewhat pessimistic, insofar as the financial model uses the same equipment for the agricultural residues as it uses for the CAM plants, whereas in reality a different choice of equipment is likely, with larger machinery operating at lower cost. Nonetheless the broad conclusions will be valid regardless of equipment choices.

If cost reduction of AD is the objective, then there are two possible areas to focus on. Approximately 65% of the cost of constructing and AD plant is in the civil works and machinery to operate the plant, and 27% is the engines and generators.

Cost reduction for engines will likely come from market developments rather than technical developments. Consider that the retail price of a 1MW Cummins engine powered diesel generator set is around $150-$200 per kW whereas the biogas engines at Gorge Farm cost over $700 per kW yet the complexity of the two units is not dissimilar. It is likely that the cost differential arises because of the relatively low volumes sold.

A more significant target for technical development is the AD process itself because it forms the largest part of the cost and is arguably the least understood. Once the cost of AD comes down, volumes of biogas engine sales will increase, and thus the cost will be brought down by a combination of competition and higher sales volumes.

Most of the world's anaerobic digesters of solid material such as agricultural residues involve large continuously stirred reactors (CSTRs). The volumes of water are very substantial and residence times are long; 40 days is not unusual. Thus the power density of an AD plant can be very low if measured in tank size per unit of power generated. The Gorge AD plant has a specific power factor of 0.23kW per m^3 of tank capacity. On the basis of 1.2 MWh per dry tonne of feedstock, this equates to 4.6 kg of feedstock digestion per m^3 per day. Part 2 of this dissertation considers in detail the *modus operandi* of a cow. Suffice to say at this stage that a cow digests around 167.7 kg of similar feedstock per day per m^3 of rumen volume. This factor of 36 difference between ruminant digestion and anaerobic digestion is the basis for presuming that the cost of the AD process can be significantly reduced.

Figure 16 and Figure 17 in Chapter 3 illustrate how much reduction in AD cost would be needed to make the technology economically attractive for agricultural residues and CAM plants respectively. In all cases a 60-70% reduction would shift the economics from poor to attractive.

Without detailed designs it is difficult to have hard and fast estimates of the specific power output per m^3 of tank size that would be needed to achieve this cost reduction. If a new generation of AD plants were to be of roughly the same complexity as the current generation, then simply doubling the throughput of an existing AD plant would halve the capital cost. In reality this is unlikely to be sufficient. For the purposes of this exercise, therefore, an increase in the specific power factor to 1 kW per m^3, an improvement of just over four-fold, is perhaps a more realistic target. Meeting this target would satisfy criterion b).

Even if criteria a) to d) are satisfied, AD must also satisfy criterion e) of sustainability. Chapter 4 sets out how AD could contribute to sustainability by using the by-products of AD to produce high value food and charcoal. The value these by-products create comes in several forms:

- By adding value to the product of AD they can help offset the cost of the feedstock.

- By creating new food production opportunities they can contribute to the notion of “Fuel and MORE food” replacing the current perception of “Fuel OR food”
- The environmental benefits of new and sustainable sources of protein and charcoal, with little or no harmful emissions is significant.

Thus the opportunity exists to create an entirely novel integrated food and energy economy applicable in all parts of the world where crops are grown, and with particular benefits for the developing world. It could be viable at both large scale, and at medium scale

PART 2

RE-THINKING THE SCIENCE OF AD

Part 1 of this research established a target for the improvement in the power density of anaerobic digestion that would allow the technology to achieve its full potential as a renewable power source.

Part 2 examines the existing science and knowledge base of AD, and extends this to draw from knowledge of ruminants and other animals that have barely been considered as sources of AD innovation. It supplements this with some theoretical analysis and novel computer modelling of biofilm growth, to conclude that very significant improvements in power density are possible, and how these might to be achieved.

Although this section includes no experimental work other than computer modelling, it draws very heavily on the experimental work of others, in a wide variety of fields ranging from anaerobic digestion, through microbiology, veterinary science and dentistry, to draw important new conclusions from the existing data.

The section concludes with a chapter on future work. This includes the development of a new generation of laboratory AD reactor, developed as part of this research, and designed to emulate much of the functionality of a rumen.

Chapter 6 INTRODUCTION TO PART 2

Work presented in Chapter 5 concluded that a power density of around 1kW(e)/m3 was needed to make AD economic. Current plants range from about 150-300W(e)/m^3, which suggests that an improvement of a factor of 3.3 or more is needed compared to existing best practice.

Anaerobic digestion of agricultural crop waste and energy crops is usually done in large stirred tanks, often with water added to keep the viscosity low and enable stirring to take place. The solids are retained in the system as long as possible to allow the lignocellulose, which is difficult to digest, as much retention time as possible. The result is systems where the retention times of solids and liquids are often more or less the same, and can be measured in weeks or months. Furthermore the presence of large quantities of solids throughout the system mean that stirring has to be mechanical rather than using gas. This adds parasitic power costs, and requires tanks that have mechanical rigidity, abrasion resistance and a structural strength greater than is needed simply to retain the liquid.

There has been considerable debate about the rate limiting steps in anaerobic digestion. Lawrence proposed that "the rate-limiting step be defined as that step which will cause process failure to occur under imposed conditions of kinetic stress" .[113] A number of authors consider, in the case of the digestion of insoluble lignocellulosic substrates, that hydrolysis is the rate limiting step.[114,115] However a more relevant parameter in this analysis is, perhaps, the commercially limiting step. To paraphrase Lawrence, "the commercially limiting step might be defined as that step which will cause failure to occur under imposed conditions of increased feeding rates". Failure in this context includes process failure, operational failure (e.g. increased viscosity leading to failure to stir) and economic failure (e.g. lower recovery of energy from the feedstock resulting in higher costs of generation).

Whilst this definition of 'limiting step' is applicable to a plant that has been constructed, where little can be done to change the nature of the operation, it fails to encompass the design alternatives available at the pre-construction stage. In any plant in which the chain of degradation processes can be separated to a meaningful extent, the limiting step becomes that which is most expensive per unit of energy produced, bearing in mind the requirements to not

fail as a biochemical process, or as a mechanical process. This 'design limiting step' offers a better target for process optimisation, as it focuses the emphasis of research and development on the step most likely to improve the overall system economics.

Although methanogenesis from VFAs is a complex process, the substrate is a suite of simple molecules in solution. If solids can be separated from the main methanogenic step(s) then methanogenesis faces none of the complexities of digestion of recalcitrant insoluble materials (cellulose) on insoluble surfaces laced with inhibitors (lignin) that create engineering limitations on design and feeding rates. Methanogenesis of clean liquids becomes amenable to gas stirring in large flexible bags if time and volume are needed. Thus low cost fabric bags could be used in place of reinforced concrete for liquid only digestion. These are available pre-fabricated with storage capacities up to 7000 m^3 ($170,000 installed) [a]. In principle any reasonable design can be made, and undoubtedly there are marketing margins to negotiate which could bring costs down even further. For reference, the 1.8MW AD plant in Kenya has a main digester of 5000 m^3 built of reinforced concrete and cost - together with stirrers, foundations, design, and ancillary elements - around $1.0 million or $200/$m^3$. The bag is 12% of the cost of the concrete tanks.

Research Focus

The analysis above suggests that hydrolysis is not only the rate limiting step, but insofar as it constrains plant design, it is also the 'design limiting step'. An alternative to conventional AD might be a system in which the solids are solubilised in a vessel or vessels optimised for the purpose, and operating at as high a rate and as high a volumetric efficiency as possible, to produce a VFA rich solution free from solids which can be digested easily and quickly. This research therefore focuses on the problems of high speed hydrolysis – with the recognition that follow-up work on downstream processing of the resultant VFA rich solution will be necessary.

An indication that high rate hydrolysis is possible comes from an experimental reactor built by Mumme et al.[116] This was a high rate Updraft Anaerobic Solid State (UASS) reactor, coupled to a

[a] For example bag tanks from www.albersalligator.com cost €30,000 for 1 000 m^3 simple bags with multiple entries and exits, or $120,000 for 7 000 m^3 shaped bags which require some modest earthworks ($50,000 estimated cost) leading to an installed cost of about $24/$m^3$.

downstream anaerobic digestion cascade. The reactor was run for 68 days and was reported to have achieved a loading rate of 17 g VS/L/d[b] with an overall efficiency of hydrolysis of solid material of around 83%. *Prima facie* this is equivalent to 860 W(electrical)/m^3 though the figure is somewhat misleading as, although the UASS itself produced mostly biogas at low loading rates of around 7g VS/L/d, as the OLR increased it produced much higher levels of VFAs, and a slight decrease in the levels of CH_4. The VFAs were then digested in the cascade of downstream digesters. In effect, therefore, this reactor is a high speed hydrolyser rather than an anaerobic digester.

Evidence that even faster hydrolysis is possible comes from ruminants. Dado and Allen looked at the feed and rumen characteristics of 12 cows fed a range of high and low fibre diets, and with and without bulk rumen fill.[117] The results relevant to this research are those of the high fibre, no bulk fill diet. These results, and the calculations of power density that flow from them, are summarised in Table 12 below.

Table 12 - Power density calculations for a cow

Daily intake	**18.7**	**Kg/day/cow dry matter**
Rumen volume (including gas headspace)	**111.5**	**litres**
Electrical energy generated/kg dry matter from commercial AD	1.2	kWh/kg before accounting for parasitic loads
Daily digestion per m^3	167.7	Kg/day/m^3 dry matter
Daily electrical equivalent	201.2	kWh/day/m^3
OVERALL POWER DENSITY	**8.4**	**kW(e)/m^3**

Very little energy is left in cow manure, probably similar to the amount of energy left in AD digestate. Assuming the high fibre diet has much the same composition as maize silage, the biogas potential would be around 300m^3 CH_4/dry tonne, equivalent to 3.1MWh thermal.[118] Assuming an engine and generator efficiency of 40% leads to a potential of around 8.4kW

[b] The paper refers to the Volatile Solids Loading Rate, but then introduces a possible element of confusion by referring to the VS-OLR. It is presumed that this does not refer to the OLR measured in grams of COD.

electrical/m^3 [kW(e)/m^3] Achieving this would represent a 28x increase in the potential power density of an AD plant compared to existing best practice.

The remainder of Part 2 considers the state of knowledge of lignocellulosic hydrolysis.

- Chapter 7 focuses on bacteria, enzymes, and their action and inhibition. Most AD literature would appear to be focused on this level of organisation and Chapter 7 reviews the literature in this field.
- Chapter 8 then considers the process of hydrolysis from the perspective of a classic ruminant (a cow) and draws some comparative data from kangaroos.
- Chapter 9 examines the problems of mass transfer that might affect hydrolysis, specifically in the formation of biofilms, and the constraints of boundary layers and diffusion.
- Chapter 10 examines the results of some simple mathematical modelling of biofilms on lignocellulose, to help understand the relevance of different parameters.
- Chapter 11 then discusses of the extent to which learning from ruminants might guide the development of high rate AD. It concludes by presenting a number of ways in which AD might be upgraded to Advanced AD and capture some of the massive benefits that appear to be available by learning from ruminant digestion.
- Chapter 12 sets out a program of future work, including the design of a potential new AD reactor able to emulate some of the digestive features of a ruminant.

Chapter 7 BACTERIA AND ENZYMES – A REVIEW

This chapter reviews the literature relating to AD with an emphasis on the cellulolytic bacteria and their enzymes, and the factors that might inhibit or promote their activity. It is confined to the existing literature and orthodoxy, rather than drawing on the literature that will be covered in subsequent chapters relating to animal models of cellulolysis.

7.1 BACKGROUND ON CELLULOLYTIC ENZYMES

Plants are complex structures, with cell walls made up of cellulose in various forms, interlinked principally with lignin, hemicelluloses, and pectins. Of these, lignin is widely recognised as being inaccessible to most digestive processes. Cellulose and hemicellulose are substantial components of the plant and although indigestible by any vertebrate, they form a major component of the food of grazers such as ruminants and macropods, which access the nutrient value by using bacteria, and possibly fungi and protozoa, to convert them to short chain fatty acids that can be absorbed and digested.

Cellulose is hydrolysed by a variety of cellulases which are expressed by both aerobic and anaerobic bacteria and by fungi. Although there are many forms of cellulase, they may be categorised into 11 discrete families.[119] The cellulases in each family share a common protein fold, and in total there are 7 recognised protein folds.

All the cellulases are made up of two components; a catalytic domain, and a cellulose binding module which helps the cellulase attach to cellulose. Within each family cellulases may be endo- or exo-cellulases. Exo-cellulases are processive, moving along the cellulose molecule after each cleavage. All exocellulases have their active site in a tunnel through which the cellulose is passed and cleaved one cellobiose unit at a time. Although this may be a disadvantage in initial positioning with respect to the target molecule it may be an advantage in locking the enzyme to the target once it is established in place.

Endo-cellulases have their active site in a cleft rather than a tunnel so that they can bind to a cellulose molecule at any point along its length, and cleave it at that point. This leaves them more vulnerable to falling off the active molecule, and also requires most of them to re-establish

themselves on a different molecule or site once they have cleaved their target. Only a few endo-cellulases are processive. Figure 21 illustrates the structure of typical exo- and endo-cellulases

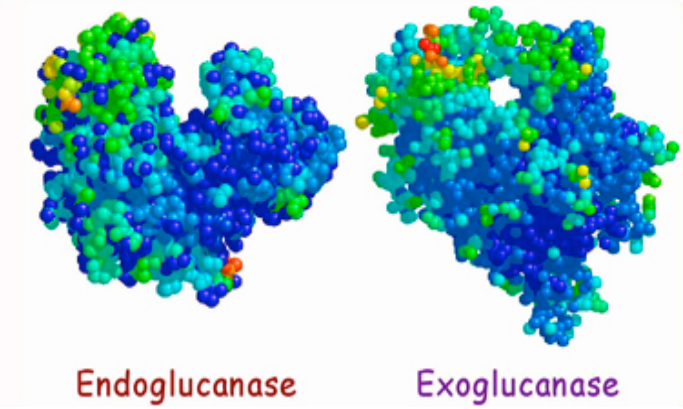


Figure 21 - Typical cellulase structures (Image from Weizmann Institute of Science)

The two types of cellulase work together synergistically, with endo-cellulases creating open ends that exo-cellulases can then attack [120].

Hemicellulases also occur in exo- and endo- forms, and are similarly attached to binding modules.

In addition to free enzymes, certain cells produce agglomerations of several types of enzyme attached via a dockerin:cohesin unit to the cell itself (Figure 22).[121] These units have the benefit of ensuring that all the relevant types of cellulase, and possibly other enzymes such as hemicellulases, that might be needed are held together. Further, the catabolites are kept very close to the cell rather than risk being lost to the general solution. The cellulase sub-units in the cellulosomes all belong to the same families and share the same basic structures as the free cellulases.

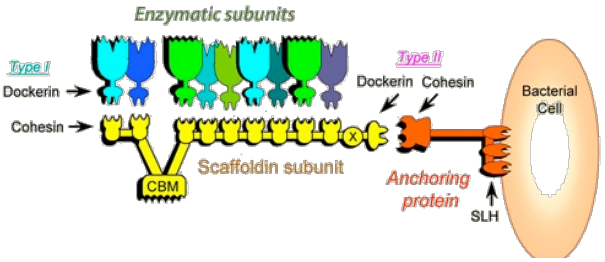


Figure 22 - Cellulosome structure. (Image from Weizmann Institute of Science)

According to Wilson it is likely that the ultimate limitation on the rate of hydrolysis of cellulose will be the speed at which cellulases can become properly attached and oriented to their target cellulose molecules, rather then the speed at which cleavage subsequently occurs.[119]

7.2 FACTORS THAT INFLUENCE THE SPEED OF HYDROLYSIS

BACTERIAL CONCENTRATION AND ENZYME EXPRESSION

There are two important factors associated with bacterial concentration.

The first is the simple observation that enzymes are produced by bacteria, so in the absence of any other confounding feature and with unlimited food, the more bacteria there are the more enzyme should be produced. This will of course be affected by other factors that may inhibit or promote enzyme expression.

Han et al. demonstrated that cellulosome expression is reduced in the presence of short chain sugars.[122] Zhang and Lynd also showed that cellulase expression (both free and cellulosomal) in *Clostridium thermocellum* was inhibited by high concentrations of cellobiose, and promoted by high concentrations of cellulose.[123] This reflects the logical observation that, in the presence of a high value food such as short chain sugars, the organism has little to gain, and much to lose, by the production of large molecular weight and energetically expensive enzymes. Thus, bearing in mind previous comments about the inhibitory impact of cellobiose on cellulase, the evidence suggests that both cellulase activity and cellulase expression are impacted by the presence of cellobiose.

The other important effect of bacterial concentration is the rate of removal of glucose from the solution.[124] Given the impact of glucose as an inhibitor of both cellulase, and β- glucosidase, and the consequent role of cellobiose as a further inhibitor of cellulase, rapid removal of glucose from the system seems to be a key pre-requisite of rapid solubilisation. High bacterial concentrations or highly active bacteria will increase the rate of absorption of glucose, and thus limit inhibition.

A further consideration is that bacterial concentration will be a function of replication rate, lysis and washout. Rumen bacteria, a frequent source of inoculum for AD plants, are noted as lysing easily.[125] Although it is not obvious why suicide is an adaptive feature for a bacterium, lysis may be a critical strategy for releasing nutrients from the bacteria back to the host ruminant, thus ensuring the better survival of the community and thus the particular bacterium's genetic material.

In an AD plant the same may be true – an unlimited build-up of bacteria would result in undesirable sequestration of biomass that could be used to make biogas. It would also result in ever increasing viscosity – making operation difficult. Lysis, at an appropriate rate, is therefore desirable. However rapid lysis means that maintaining the bacterial population requires strategies to minimise washout, and excessive lysis could cause community collapse. The factors that dictate the rate of lysis seem not to have been well investigated. In at least one case (*Fibrobacter succinogenes*) depleted levels of extra-cellular sugar causes inactivation of the autolysins, and thus reduces cell turnover levels.[125] Intriguingly, this suggests that lysis increases in the presence of excess sugars. This may be relevant to establishing a rate limitation for AD in environments where sugar removal is less rapid. It reinforces the notion that removal of sugars from the hydrolysis stage as fast as possible is perhaps an important feature of rapid hydrolysis.

Adhesion, Adsorption and Detachment

The molecular weight of glucose is 180. The molecular weight of a cellulase molecule may be of the order of 30,000-70,000.[126,127] Thus each cellulase molecule must liberate, and deliver to its manufacturer, 200-500 glucose molecules simply to break even on a mass basis. From an evolutionary perspective it seems unlikely therefore that a bacterium would expend substantial energy to produce a cellulase molecule, only to release it to float free in the water.

Micro-organisms appear to adopt two strategies to avoid losing control of nutrients that they have catalysed. The first is to form a cellulosome, in effect a massive complex of enzymes that contains all the components needed to catabolise cellulose.[128] The large size of the cellulosome/bacterium complex (molecular weight estimates range from 2MDa to up to 100MDa[129]) might be expected to limit access to cellulose occluded by lignin or other structural chemicals. However the multi-functional nature of the cellulosome seems to enable it to degrade a range of substances – presumably thus getting access to more cellulose.[119] The second strategy is for a bacterium to adhere to a molecule of substrate and express its cellulase at that point.[130] It is quite probable that some bacteria use both strategies.

Further evidence for these strategies comes from work on animal rumens suggesting that 88%-91% of ruminal endoglucanase and xyloglucanase activity derives from microbes attached to

feed particles.[131,132] In the rumen, most bacteria are reported to attach themselves to plant fibres by expressing glycocalyx, with up to 75% of the total bacterial population becoming attached.

Another strand of evidence comes from the investigation of the cellulolytic system of *Trichoderma reesei.*[133] This showed that effectively all cellulase is bound rapidly to lignocellulose biomass. Intriguingly, some organisms that express hemicellulase are also observed to adhere to cellulose, rather than hemicellulose.[119,129] In the case of *Thermobifida fusca* the xyloglucanase produced is attached to a cellulose binding module rather than a hemicellulose binding module. This makes sense because, although *T. fusca* is unable to metabolise xyloglucan catabolites, it presumably needs to remove hemicelluloses from the cellulose as a pre-cursor to cellulolysis. Thus it only has an interest in degrading hemicellulose molecules that are attached to cellulose.

The speed of bacterial attachment to the substrate can be high, with attachment starting within a minute of exposure to Avicell, and peaking at times varying from 30 minutes to 24 hours.[131] This suggests that the levels of both cellulolytes and cellulase in free solution are of limited relevance to the rate of hydrolysis, but it also raises a question about how this information reconciles with O'Sullivan's observations on the rate of surface colonisation of cellulose by cellulolytes.[123]

Figure 23, reproduced from O'Sullivan's paper shows the development of a colonies of cellulolytic bacteria on 50μ particles of cellulose at a range of times following inoculation.[134] It shows clearly that colonisation of the particles takes many days, and only by day 41 is the cellulose completely colonised. It also shows that biofilms become thicker and more complex over time – ultimately binding together numerous smaller particles into large clumps.

Adhesion or adsorption is possible whilst there is ample substrate but as hydrolysis proceeds the substrate is consumed and the suitable surface area will decrease whilst the bacterial population and the total amount of enzyme increases. Ultimately this must either cause bacteria to detach from the surface, or lyse. Both actions will result in bacterial loss (by flushing or death) in many conventional AD reactor designs. Likewise, enzymes must also become detached from the substrate, and may either be destroyed by other organisms or lost by flushing out. However their fate seems largely dependent on the presence of lignin.

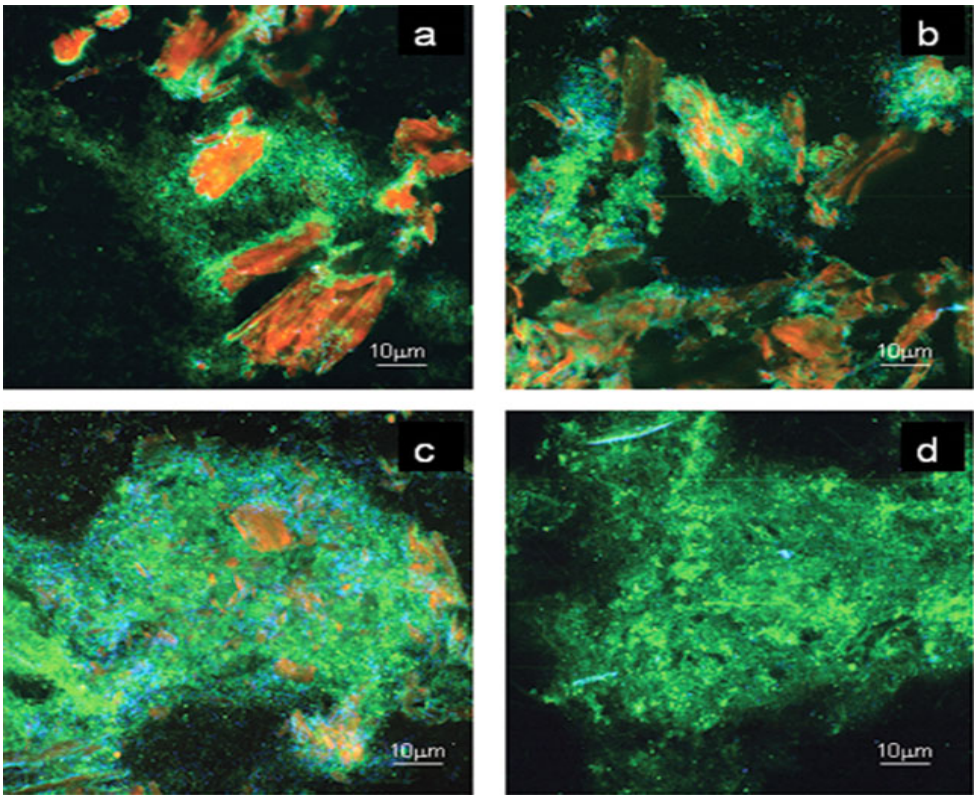


Figure 23 - Development of bacterial colonies on cellulose. Green and blue dots are cellulolytic bacteria, the red is unoccupied cellulose. (a) is day 1, (b) is day 5, (c) is day 25 and (d) is day 41 when cellulose availability finally becomes limiting. (*Images reproduced from O'Sullivan 2005*).

THE ROLE OF LIGNIN

Lignin is not easily catabolised by any bacterial enzymes and there is a strong negative correlation between lignin content and the ultimate biogas potential of AD feedstocks[50].

Xu and Ding demonstrated that, whilst cellulase was desorbed from the solids as the hydrolysis of pure cellulose progressed, this was not the case in the presence of lignin.[133] With natural lignocellulose substrates, little if any cellulase was desorbed; it all appears to be re-adsorbed by the lignin. Rahikainen et al. demonstrated that cellulase adsorption onto lignin causes the cellulase to become de-activated, possibly due to denaturing at hydrolysis temperatures.[135]

Börjesson and Tjerneld demonstrated that the level and speed of conversion of lignocellulose could be significantly enhanced by the addition of relatively low concentrations of polyethylene glycol (PEG).[47] Hydrolysis of lignocellulose in the absence of bacteria, that has halted as a result of low levels of enzyme, can be restarted either by the addition of fresh enzyme or by the

addition of enhancers such as PEG 4000. It is hypothesised that this bonds to the lignin via hydrogen bonding and hydrophobic interactions – thus displacing cellulase.

PEG displacement of enzymes is highly temperature dependent, and most effective in the range of 40C to 50C. Concentrations of the order of 2.5g/l (with substrate concentration of 50g/l) are suggested to enhance cellulolytic activity by of the order of 100%. The problem with this approach is simply the cost of PEG. (±$1,500/tonne). This would add $75/tonne of feedstock to the cost of hydrolysis to produce electricity whose total value is only $150/tonne. On the other hand Borjesson et al. noted that PEG adsorption on lignin at 20C was 50% of the level at 50C.[47] This suggests that moderate cooling of the undigested solid fraction following hydrolysis might release a substantial quantity of the PEG for re-use.

Because lignin is recalcitrant and bound intimately into the structure of cell walls it is surmised that a second possible reason for lignin inhibition, other than cellulase de-activation and adsorption, is simply physical occlusion of potential hydrolysis sites.

ENZYME CONSERVATION AND RE-USE

In principle, an AD plant could benefit from the recycling of enzymes from liquid digestate into the feedstock. Knutsen et al. looked at the possibility of retaining and reusing hydrolytic enzymes in the enzymatic hydrolysis (i.e. no bacteria present) of pre-treated corn stover.[136] They considered two approaches; simple vacuum filtration at around 20-25µ, and then vacuum filtration followed by ultrafiltration. They observed that saccarification was enhanced by even the coarse vacuum filtration, with the retentate being returned to the reaction vessel together with additional substrate. On the other hand, vacuum filtration followed by ultra-filtration of the permeate from the first filtration stage had little additional effect on the rate or amount of cellulolysis compared to simple vacuum filtration. They reasoned that this is because all the enzyme remains bound to the fine particulate matter, and little if any active enzyme is present in the liquor. This reinforces the observations described previously of the binding of cellulase and bacteria to plant fibres in the rumen, and has important implications for the design of any high speed reaction system.

TEMPERATURE AND ENZYME DEGRADATION

Enzyme activity is temperature dependent. However enzyme stability is also temperature and pH dependent, and the temperature and pH of maximum activity are not necessarily those of maximum stability.

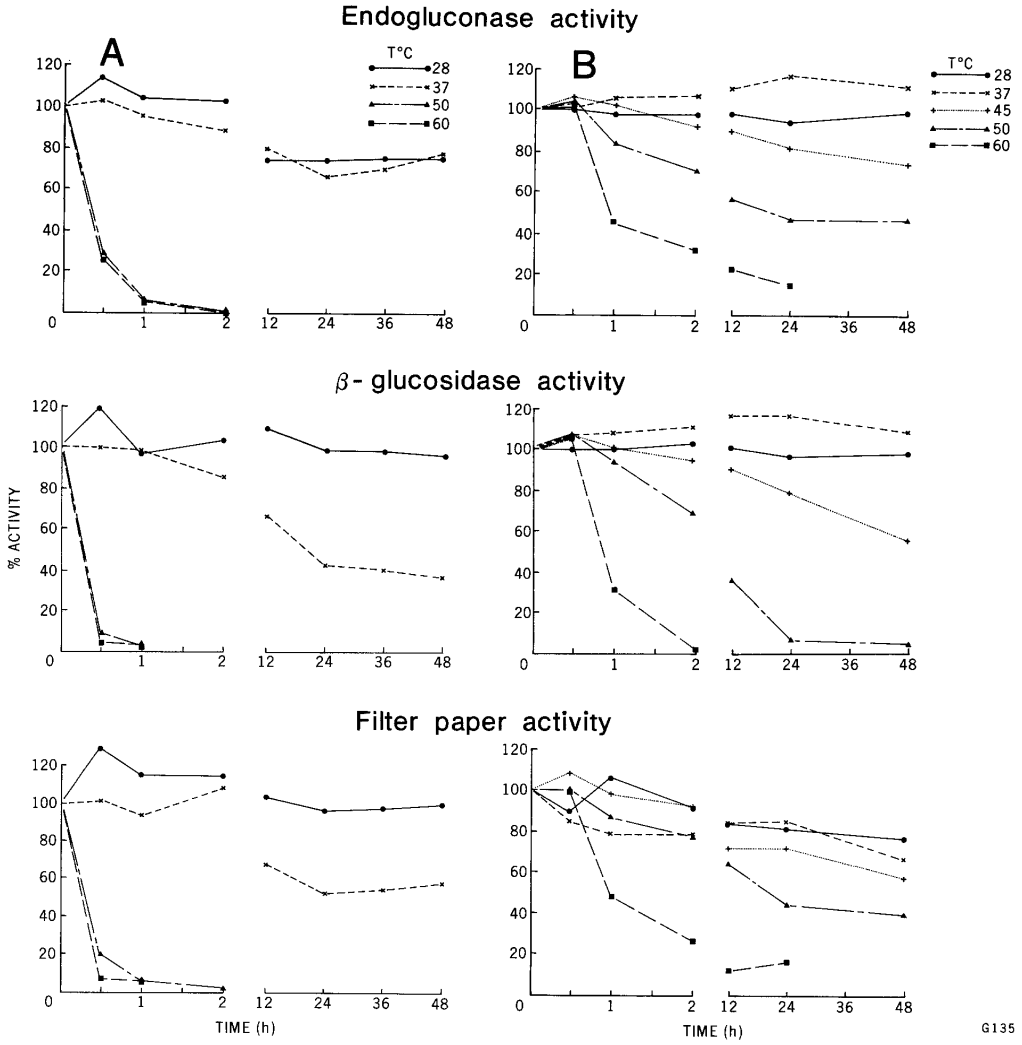


Figure 24 – Thermal stability of the endo-gluconase, β-glucosidase and filter paper activities of *T. harzianum* E58 culture filtrates which were either (A) used directly or (B) were adjusted to pH 4.8 prior to incubation. (*Graphs copied from Sadler 1985*).

Saddler et al. compared the enzyme systems of *Trichoderma reesei C30* and *Trichoderma harzianum E58* at temperatures ranging from 28°C to 60°C and at pH between 4.6 and 6.[137] They observed that both cellulase and β glucosidase were more or less stable at temperatures below 45°C (Figure 24), but that above this temperature the activity declined very rapidly at a pH of 6. At a pH of 4.8 the rate of decline at higher temperatures was markedly lower, albeit still high.

Although these enzymes were of fungal origin, it is not unreasonable to expect similar patterns to exist amongst the enzymes produced by ruminant bacterial flora. This has implications for reactor design. Reactors designed to retain or recirculate enzymes may well need to consider the trade-off between temperature stability and temperature dependent activity.

MECHANICAL AND THERMAL TREATMENT

Several groups have reviewed the range of pre-treatment options to enhance the solubilisation and hydrolysis of lignocellulose.[138–140] Many of the options they considered however require either bulk or expensive chemicals. As the objective of this research is to develop cost effective Anaerobic Digestion – able to operate economically at both large and small scales, and as much of the opportunity to use the technology will be in remote or developing world sites, treatments requiring such chemistry are not being considered. Likewise, high pressure steam treatments such as steam explosion are not being considered as the complexity of operation that they would add is unlikely to be justified for small scale plants.

This largely limits treatment options to mechanical methods such as milling to reduce particle size. Other possibilities exist, such as low temperature and pressure water treatments, and possibly treatments with ultrasound, as all of these can be delivered realistically in the operational context envisaged but they are not considered here.

The size of substrate particles will determine the surface area accessible to micro-organisms, and so, it is widely assumed, the rate of hydrolysis. Several groups have examined the effect of particle size on hydrolysis rates and gas yields.[141,142]

Sharma studied the rate of methanogenesis over a period of 8 weeks from a number of different plant materials, at a range of different particle sizes.[141] The samples included three grasses; wheat straw (*Triticum aestivum*), rice straw (*Oryza sativa*) and dhub grass (*Cynodon dactylon*). The data from Sharma is particularly relevant as it was based on straw rather than food waste. Data for the digestion of the three grasses has been re-analysed in Figure 25. The grasses showed almost identical responses to particle size reduction, so the data shown represents the arithmetical average of all three.

This data demonstrates a clear relationship between particle size and both the rate and quantity of biogas production for all particle sizes examined between 0.088 and 6.0mm, and then large unshredded particles at around 30mm in length. No benefits in total gas yield or digestion speed were seen below 0.4mm, and little benefit below 6mm. However there were substantial differences between the unshredded particles and the 6mm particles – of 30% in some instances.

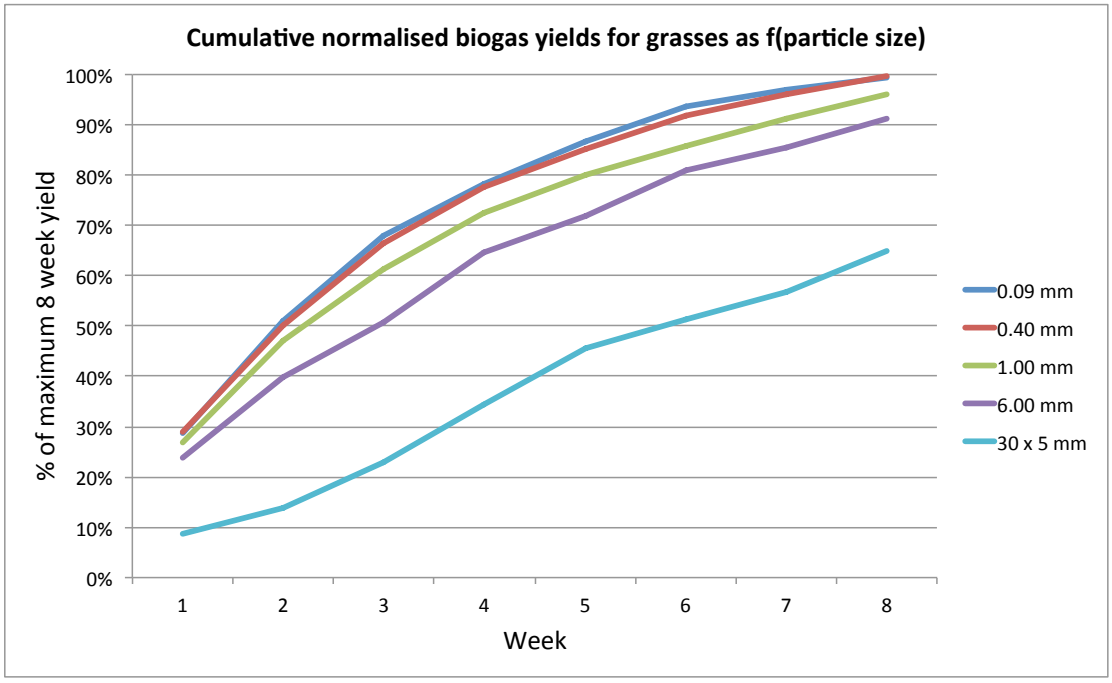


Figure 25 – Gas yields as a function of time for a range of grasses and leaves, showing diminishing returns as particle size decreases. *Data reproduced from Sharma.*

The experiments showed a material difference in gas yields at 8 weeks between 6mm particles and the 0.4mm particles, though this was only around 5%-10% of ultimate gas yield. Likewise for the rate of gas production – the main gains came from the reduction to 6mm. Thereafter gains were more modest.

Although comminution has benefits, it also incurs energy costs – the smaller the particle the larger the cost. Cadoche and Lopez examined the energy costs of comminution of a variety of feedstocks with a variety of mills.[143] Their results are presented in Figure 26.

Figure 26 suggests that agricultural wastes, as characterised by straw and corn stover, could be reduced to 3 mm particle size with only 20kWh/tonne of electricity, and even around 2 mm should be possible with under 30kWh/tonne. Bearing in mind that a tonne of dry biomass generates around 1.4MWh of electricity, this is around 2% of total energy content. Thus, *prima*

facie modest particle size reduction is an affordable part of the strategy for high rate hydrolysis of agricultural wastes though its utility is questionable.

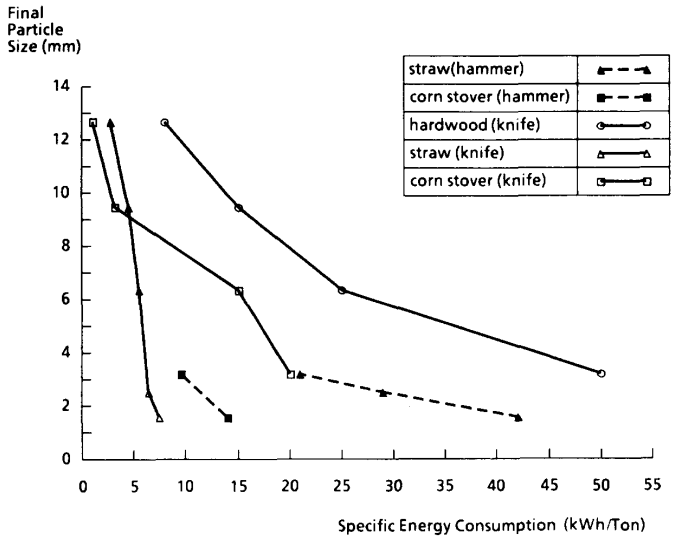


Figure 26 - Energy consumed in comminution of a range of grasses as a function of particle size (Graph copied from Cadoche[143]).

INHIBITION

The inhibition pathways affecting hydrolysis are complex. The inhibition occurs in two principally different forms; product inhibition of enzymes, and VFA/pH inhibition of the cellulolytes themselves.

Enzyme Inhibition by Cello- and Xylo-oligomers

Cello-oligomers and other sugars are well known inhibitors of enzymatic hydrolysis. Oh et al. studied the effects of glucose on the activity of cellulase and β-glucosidase (cellobiase).[144] They showed that glucose concentrations of 10g/l reduced the activity of cellulase by 50%, and that cellobiose concentrations of 10g/l reduced the initial rate of hydrolysis by 70%. Furthermore, glucose concentrations of 10g/l reduced the activity of β-glucosidase by almost 90%.

Holtzappple et al. also demonstrated that glucose and cellobiose inhibit cellulase activity, and that the binding constants for cellobiose are 3-14 times as great as those from glucose.[145] They postulate that the inhibition is competitive, even though it exhibits the characteristics of non-competitive inhibition. They attribute this to the insolubility of cellulose, and thus the low diffusion potential, which means that cellobiose is free to bind to the active site in any cellulase in

solution. Further confirmation of this hypothesis comes from the argument that the structural similarities between cellulose and cellulose oligomers are high, and that cellobiose has more binding sites than glucose. Thus if it is engaged in competitive inhibition the binding constant should increase as sugar length increases. This accords with the higher binding constants measured for cellobiose.

As β-glucosidase converts cellobiose to glucose, low concentrations of glucose which are not taken up by organisms or removed by diffusion could result in a very strong inhibitory chain of events. Thus together, the work of Oh and Holtzapple demonstrates the complexity of the feedback loops governing cellulose degradation.

Other sugars have similar effects. Xylose and xylan have been shown to inhibit cellulase activity severely.[146] Although the study could not identify which of the xylo-oligomers contributed what degree of inhibition, a mix of xylo-oligomers at concentrations of 12.5g/l were demonstrated to inhibit the rate of hydrolysis of cellulose (2% Avicell) by over 80% initially, and to reduce ultimate yields by 38%. At much lower concentrations, 1.67g/l, the effect was lower but significant; ultimate yields were down around 13% (from 81% to 68%) and the yield after 24 hours was approximately 50% of that from the control. This study was done with exogenous enzymes, rather than enzymes produced by bacteria within the culture – demonstrating that this effect, at least, is not related to the expression of the enzymes or the activity of the bacteria.

Kumar and Wyman demonstrated that cellulase inhibition at 1% (10g/l) cellobiose concentration was around 12% and that cellulase binding can be completely shut down by cellobiose at concentrations of around 150g/l.[147] At lower concentrations the effect could not account for all the deactivation seen by Oh. By their estimates, Oh's data showed 75% loss of hydrolytic activity at 20g/l of cellobiose, but their own work on adsorption could only account for 15% - thus implying that adsorption was not the only mechanism at play in reducing cellulase activity. Kumar and Wyman also demonstrated that binding could be reversed by increasing the concentration of substrate.

Knutsen et al., in a study of the adsorption properties of enzymes and their recovery from reactions by filtration and recirculation, also studied the effect on their reactions of removal of

the short chain sugars.[136] Although the concentration of these in their reaction vessels was reduced by filtration (from 70mg/l to 10mg/l) there was no measurable improvement in the rate of hydrolysis. They reason that this is because the concentration had not been lowered sufficiently to reduce inhibition. However by their reckoning, further reduction of the concentration would render the ultra-filtration permeate too dilute for economic processing to ethanol.

Qing and Yang also compared the relative inhibitory effects of cellobiose and glucan derivatives with xylose, xylobiose and xylo-oligomers.[146] They demonstrated that whilst cellulose catabolites had very strong inhibitory effects in the first 10 hours of hydrolysis, thereafter inhibition was dominated by the hemi-cellulose catabolites.

It seems likely, therefore, that the equilibrium between the hydrolysis products of cellulose and hemicellulose and the expression and activity of cellulase is a key factor in regulating the rate of hydrolysis. Systems that seek to disturb this equilibrium by removal of these catabolites may offer a promising approach to accelerating the solubilisation of biomass.

The role of VFAs

Siegert and Banks examined the effects of different initial VFA concentrations independently of pH on the mesophilic hydrolysis of paper (principally cellulose with around 1-1.5% lignin) and the fermentation of glucose.[148] Both experiments were batch experiments controlled at a pH of 7.

They concluded that initial VFA levels of around 2g/l and above both reduced the production of proteins, presumably enzymes, and reduced the destruction of cellulose substantially. Parallel experiments using glucose as the substrate instead of paper showed only a minimal reduction in VFA production up to an initial VFA concentration of around 6g/l whereupon it too started to fall rapidly. It seems that the VFA concentration acts to diminish the ability of the cellulolytes to synthesise enzymes, and thus hydrolyse, to a greater extent than it diminishes the ability to digest glucose and produce VFAs. This disparity in inhibition between the production of enzymes and the digestion of glucose may incline systems operating close to their maximum VFA concentration to become unstable. VFA inhibition of hydrolysis would not prevent the stock of glucose in solution continuing to be digested, and creating more VFAs.

VFAs are removed by methanogenesis, so it is relevant to explore the constraints on methanogenesis created by VFAs. Pullammanapallil et al. demonstrated that propionate, even at high concentrations, did not seem to inhibit methanogenesis from glucose, and thus presumably did not interfere with acidogenesis.[149] This is somewhat perplexing given the results of others, and may perhaps be an artefact of the experimental approach used.[148] The conclusion needs further examination. In contrast, Mösche and Jördening looked at the inhibition of the acetate and propionate degradation reactions by propionate (substrate inhibition), and the inhibition of the propionate degradation to acetate by acetate (product inhibition).[150] They concluded that substrate inhibition was largely controlled by undissociated VFA concentrations, with some independent pH influence. Product inhibition was however controlled by the acetate:propionate ratio – indicating a measure of competitive inhibition. Product inhibition occurred from an acetate:propionate ratio of 1 upwards.

Consequently, any system where either VFAs are not removed from the hydrolysis reactor, or where they are removed for digestion and then propionate is recycled to the hydrolyser from the spent digestate runs a risk of propionate build-up due to acetate:propionate inhibition of the hydrolysis, whilst continued VFA production from glucose stocks adds further to the burden of VFA inhibition. This could then inhibit hydrolysis, and become rate limiting. It may also result in unstable or chaotic operation.

The role of pH

The role of pH in hydrolysis is complex; it can affect the solubilisation of lignocellulose in a wide range of different ways and there is limited clarity in the literature about the extent to which pH effects specifically affect solubilisation or the other process stages in anaerobic digestion. pH is unavoidably linked to the production of VFAs, and the separation of VFA and pH effects is complex and poorly understood. Some of the documented interactions are as follows:

- pH contributes to the stability of enzymes, with low pH prolonging and high pH shortening their half life. This has already been discussed above.
- pH affects the equilibrium position of $NH_3 \leftrightarrows NH_4^+$ which has different impacts at various stages in the anaerobic digestion process.

- Differing pH levels can produce different proportions of VFAs, and propionate can be an inhibitor of methanogenesis. [151,152]
- The degree of dissociation of VFAs is pH dependent and may impact on intra-cellular proton transport, and thus cell viability and productivity.

Veeken et al. attempted to separate the effects of pH and VFA concentration on hydrolysis of municipal solid wastes.[153] They concluded that inhibition by VFAs was not a factor – and any apparent inhibition was due to the pH effects of VFA concentration rather than the VFAs themselves. However there are two possible limitations to their conclusions.

The first is that in order to maintain VFA concentrations at any given level, effluent was removed from the circulating flow in their solid bed reactor, and replaced with fresh liquid with the same approximate electrolyte balance. This may have resulted in some relatively modest loss of bacteria and enzymes from the system, with potential consequences for hydrolysis rates.

The second, and arguably more significant limitation, is that they maintained pH by adding a strong acid or base as needed. This may substantially change the level of undissociated VFA in solution compared to the situation where all the H^+ ions were provided by the VFAs. Palmqvist et al. discuss various hypotheses for the mechanism of VFA inhibition.[154] Whilst there is some doubt as to the specific mechanism, a common theme seems to be that the undissociated form of the acid is liposoluble and therefore tends to diffuse into the cells. Because cells tend to maintain near neutral pH internally, these molecules then dissociate – causing a rise in hydrogen ion concentration within the cell. The anionic forms, however, are lipophobic and therefore do not migrate out of the cell. The accumulation increases until the pH is equal both sides of the cell wall. Thus maintenance of pH by means other than VFAs may have effects that do not relate to the real world impact of 'VFA maintained' pH.

pH also determines the $NH_4^+ \Leftrightarrow NH_3$ equilibrium. This equilibrium is an important source of buffering capacity, and NH_4^+ is critical to the health of bacterial populations and in particular to the production of enzymes needed for hydrolysis. However NH_3 is highly toxic to methanogens in particular with reported levels of tolerable free ammonia of around 150mg/l.[155] On the other

hand, NH_3 appears to be much less toxic to cellulolytic species. Fernandes et al. showed that free ammonia levels of 280-960mg/l NH_3 – N were well tolerated and did not inhibit hydrolysis.[156]

Thus overall process optimisation may dictate a modestly sub-optimal pH for solubilisation if it can be used to create more favourable conditions for methanogenesis. This is particularly true if managing the pH can help inhibit propionate production.

Summary of inhibition pathways

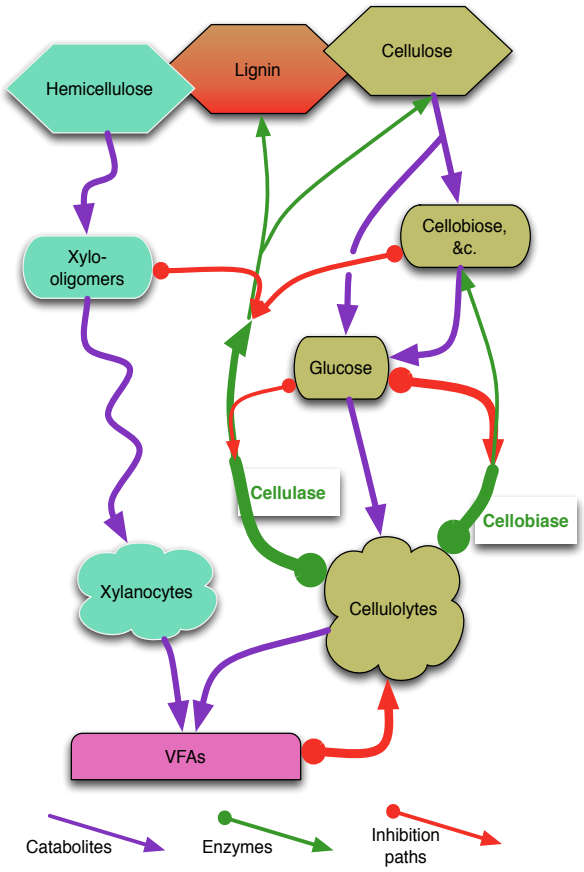


Figure 27 – A simplified diagram of the relationships between products and inhibition pathways in the hydrolysis of lignocellulose.

Figure 27 shows schematically the principal identified inhibitory pathways affecting the hydrolysis of cellulose. One might anticipate similar complexity in the hydrolysis of hemicellulose. Note that lignin is not shown as an inhibitor; it is assumed to adsorb cellulase and thus to consume otherwise useful resource.

Chapter 8 THE DIGESTIVE SYSTEM OF GRAZERS

The best anaerobic digesters in common use are ruminants such as cows. Work presented in Chapter 6 demonstrated that a cow could hydrolyse 8.4 kWe/m^3, around 28 times as fast as an AD plant. It is useful therefore to consider the hydrolysis systems in a cow as a model for what can be achieved, and perhaps to provide clues as to how high speed hydrolysis might be achieved. Further clues can be found in the digestive systems of other animals.

Kangaroos are also grazers that rely on foregut fermentation. Marsupial and placental mammals appear to have diverged around 125M years b.p. The first evidence of grass is in the coprolites of dinosaurs in the late Cretaceous – about 80 M years b.p. Thus the two groups of animals diverged some 45 million years before grass and grazing seem to have evolved, and have therefore separately evolved mechanisms for the digestion of rough grasses. Comparing the two systems to determine what is common between them, and what is different, may also provide important clues for the design of AAD.

8.1 OVERVIEW OF RUMINANT DIGESTION

Cows are a well studied group of ruminants and provide a good model for ruminant digestion. Digestion in a cow happens in a number of distinct stages:

1. Cows graze by tearing grass, chewing it and swallowing. Typical particle sizes swallowed are of the order of 1mm^3 to 10^3 mm^3.[157] The chewed material is mixed with saliva, and passed to the reticulorumen.
2. The reticulorumen has a typical volume of around 110-170 litres.[117,158] The reticulum is a distinct compartment within the reticulorumen with a volume of around 20 litres, which serves both as an entry point and an exit point for feed. Both compartments (reticulum and rumen) are formed from tissues with large, highly vasculated, surface areas (Figure 28) which absorb VFAs. In the reticulorumen the grass joins the mat of digesting material. Regular muscular contraction of the reticulorumen keeps material moving, and allows any gas that evolves to be expelled.

Figure 28 Lining of Rumen (L) and Reticulum (R) of a cow showing the high surface area available for VFA absorption. *Images from Colorado State University*[159].

3. The mat of materials is bathed around once a minute in ruminal fluid which is moved through the mat by peristalsis.[157,160] The mat itself has something of the texture of a compost heap. Hungate reports that the rumen contents tend to be of the order of 10%-18% w/v dry matter. For reference, a pile of wood chip has a density of around 140 dry kg/m^3 – around the middle of this range. This gives a sense of the solidity of the mat – it is not something that could be stirred; you cannot plunge a hand into the rumen, you have to dig your way in. Figure 29 shows the interior of the rumen of a live cow photographed through a fistula. The packed nature of the substrate can be clearly seen.

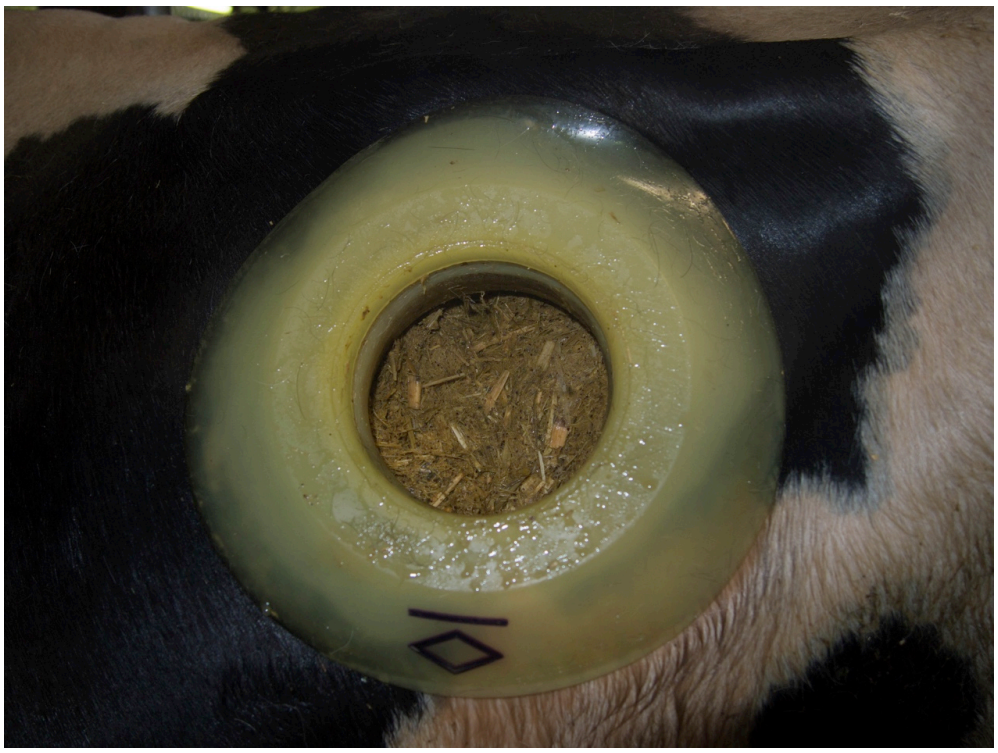


Figure 29 - Rumen contents of a dairy cow photographed through a fistula.

Periodically material from the reticulorumen is returned to the mouth as a bolus. The liquid is compressed out, and it is then chewed. Chewing the cud, or rumination, occupies up to 30% of a cow's life. In one study, 38% of the time a cow spent not idling was spent ruminating (Figure 30), so it clearly has a significant evolutionary benefit.[159]

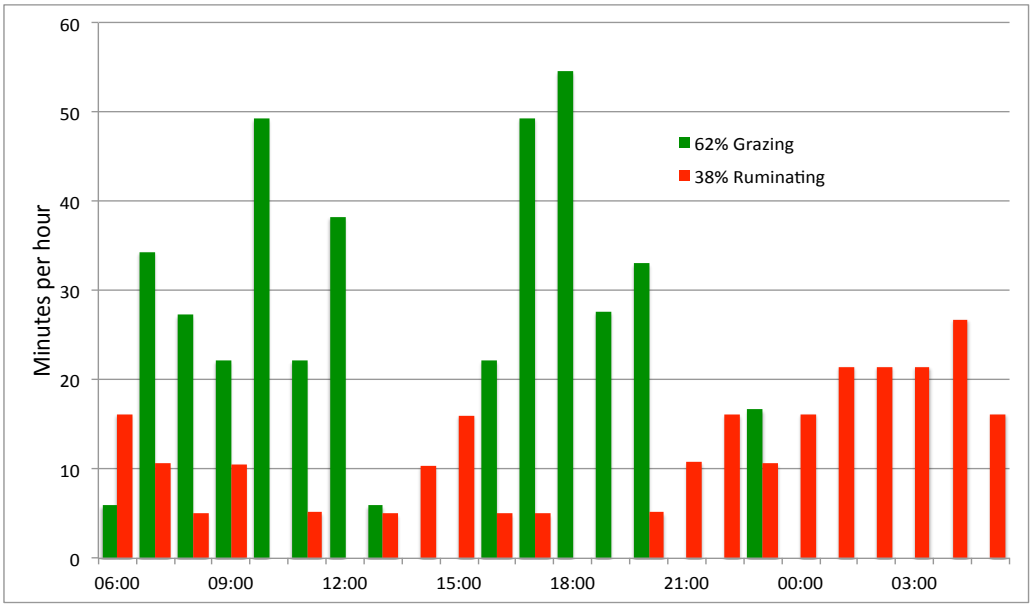


Figure 30 - Time spent ruminating and feeding – *graph recreated from Bowen (2003)*[159].

4. At periodic intervals the opening from the reticulum to the omasum opens, and small particles (<5 mm^3) flow through into the omasum. Larger particles are retained by conical tooth-like epithelial projections around the orifice.[157]
5. The omasum is a smaller compartment than the reticulorumen that also has a large surface area – it is estimated to represent around one third of the surface area of the fore-stomachs of a cow. Its function appears to be to absorb any residual VFAs in the liquid flowing through it. It also traps small particles on its large

Figure 31 – Interior surface of the Omasum, showing extensive folding and absorptive papillae. *Image from Colorado State University*.

surface. Ultimately these particles form flakes that are shed from the absorptive surfaces. The omasum possibly also absorbs bicarbonate, thus reducing the buffering capacity of the fluid and allowing the abomasum to function at a relatively low pH.

6. Finally, material from the omasum passes through to the abomasum, which is the equivalent to the stomach in a monogastric. This expresses both proteolytic enzymes and ribonuclease to recover nutrients from the bacterial population flowing through.

Figure 32 is a simplified representation of the steps in a ruminant's digestive system.

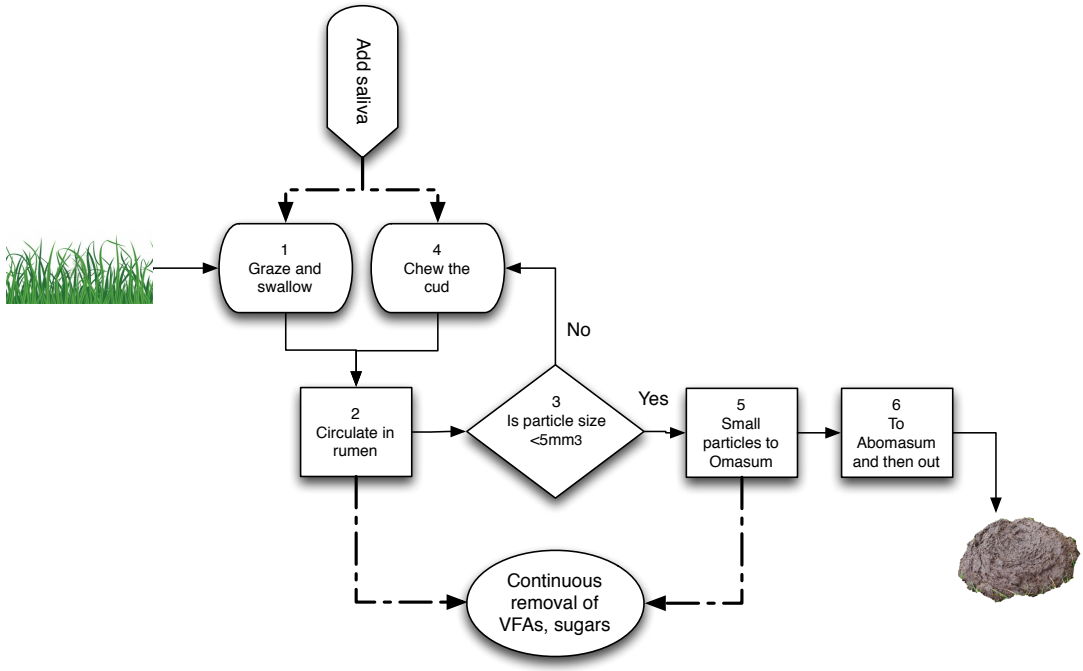


Figure 32 - Schematic of ruminant digestion system showing principal pathways from feed to manure.

RUMINANT SALIVA

Saliva from the ruminant parotid gland is a crucial part of the digestive system. An adult cow produces 50-60 litres per day[c], mostly during feeding and ruminating.[157,158,161] Parotid saliva

[c] Hungate cites 1-3 times the volume of rumen content. This is confusing, but presumably refers to dry matter content rather than total content which would be 150-170 litres. Assuming, say, 15% w/v rumen solid content, and a relative density of 1, then the rumen solid content volume would be ≈25 litres, corresponding to a saliva volume of 25-75 litres per day.

from a cow contains 100-140 milliMolar (mM) bicarbonate ions; 10-50mM inorganic phosphate; 10mM of Nitrogen as urea[157]. In addition Reid et al. reported small quantities of ascorbic acid.[161]

Cows are, of course, not the only animals that live effectively on grass. Sheep too spend their lives on grass, though they have somewhat different grazing habits commensurate with their smaller size. McDougall's work on sheep saliva shows that a modest amount of free energy is used to produce copious quantities of saliva with a pH of in excess of 8.[162] The alkalinity derives principally from HCO_3^- with the principal cation being Na^+. McDougall also notes the high levels of phosphate, and postulates that this may have a role in both buffering and in maintaining the health of bacterial populations.

8.2 Macropod Digestion

Macropods (kangaroos and wallabies) eat similar coarse grasses to ruminants. They digest these initially using microbes in a fore-stomach that has two distinct elements; a sacciform element which receives food from the oesophagus, and passes it forward to a tubiform fermenting chamber. The sacciform element acts somewhat like a continuously stirred tank reactor (CSTR), whereas the tubiform element acts more like a plug flow reactor.[163]

Beal studied the parotid gland secretions of kangaroos, and determined that they have concentrations of bicarbonate and phosphate ions in their saliva comparable to those of placental ruminants.[164] These contrast with the typically much lower concentrations from the saliva contents of non-ruminants. Beal notes that this is a remarkable example of convergent evolution, and clearly of some significance to the digestive capabilities of these animals.

Some marsupials also indulge in occasional regurgitation and remastication.[165] In macropods (kangaroos) this has not been seen as rumination, as investigators have observed that there would seem to be little need for remastication to reduce food particle size. Kangaroos chew their food well the first time through and have no intermittent opening that filters by size such as that between the reticulorumen and the omasum in ruminants. Consequently the behaviour has been given the name merycism. Its function is not well understood, but it is believed to be related to

the stimulation of salivary excretion.[165] A hypothesis to explain the different roles of rumination and merycism is discussed in 8.3 below.

8.3 Implications for Advanced Anaerobic Digestion

The following sections aim to highlight, and where appropriate compare, the important parts of the digestive systems of cows and kangaroos that might be relevant to AAD.

Rumination as a means to reduce particle size

Rumination is generally seen as a mechanism for reducing the size of feed particles, and as a way to stimulate salivation. However closer analysis suggests that this may only be a partial explanation of the purpose of rumination.

Sharma's original analysis, as discussed in Chapter 7, concluded that the reduction of particle size was important down to perhaps 1mm or perhaps 0.4mm but no further. No further conclusions were drawn. However re-plotting the data as in Figure 33 hints at other processes at work in rumination other than simply size reduction.

Figure 33 shows the rate of gas evolution over time per imputed unit of surface area for the various particle sizes. The y axis is logarithmic; the z axis is simply the ordered size categories, the scale is not representative. One might expect, if surface area was the critical parameter, a reasonably consistent rate of gas production per unit of surface area in the early stages of fermentation. As time progresses the particles should become smaller, and the rate of gas evolution as a function of the original particle size should decrease. However the plots tell a different story.

For the small particles, the rate of gas evolution per unit of original surface area decreases over time as might be expected, given the reduction of surface area with time. For the largest particles (pieces of straw 30mm long by 5mm diameter) the rate of gas evolution remains roughly constant – suggesting that either there is little loss of particle size, or that new areas of the particle are continuously being colonised by cellulolytic biofilm and that it is not surface area that is limiting but the rate of biofilm colonisation.

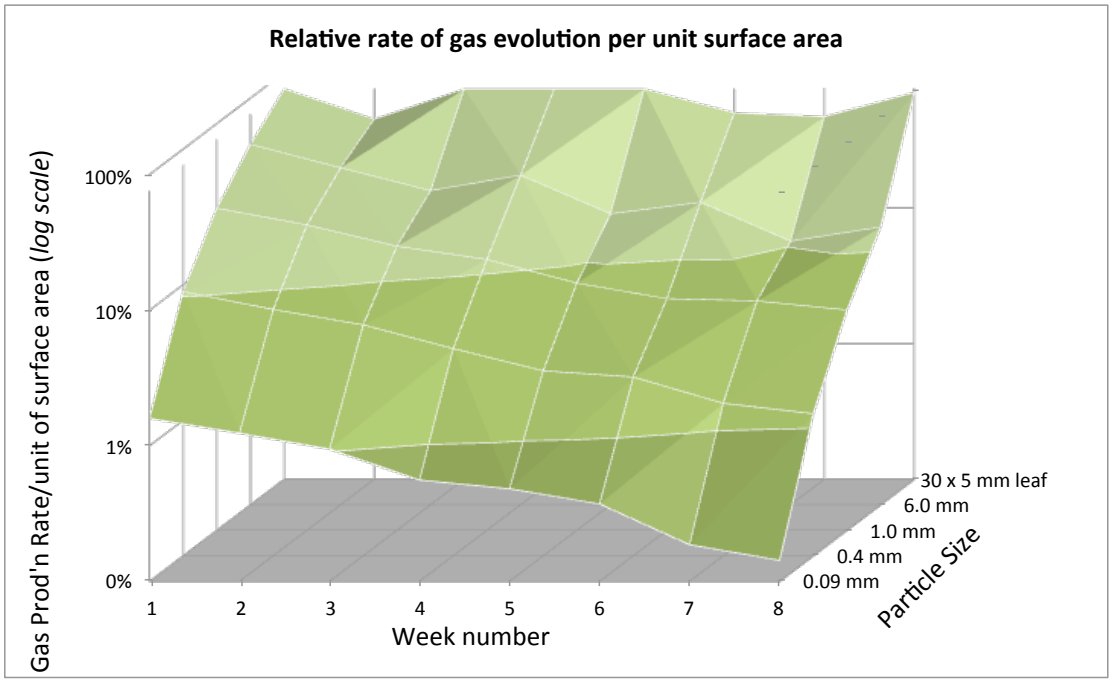


Figure 33 - Gas evolution rate per unit surface area as a function of starting particle size, plotted using data given by Sharma, but adapted to show estimated surface area of particles. The surface area is estimated by assuming particles are cubes of side equal to the nominal particle size, except the leaf particle where the assumption is a cuboid 1mm deep with other dimensions as quoted.

Figure 34 examines the same data in a different way. It plots the rate of gas evolution per unit of surface area at the start of the experiments against the relative surface area/gram of the feedstock at the start of the experiments. It shows the dramatic decrease in the rate of gas evolution per unit surface area. This suggests that, whilst increasing surface area may be an important consequence of rumination, surface area alone is not the principal determinant of digestion rate when all other factors are equal.

RUMINATION AND MERYCISM

The fundamental difference between kangaroo and ruminant digestion is in the form of the fore-stomach, and the ability to sort particles by size.

In ruminants feed particles are mixed and held in the rumen until they are largely digested, much as in a Continuously Stirred Tank Reactor (CSTR). A CSTR that is operating as a continuous flow reactor has the weakness that some portion of the most recently ingested feed will short circuit the reactor and pass out with little or no retention. The proportion that passes through quickly is directly dependent on the ratio of the size of the reactor to the feed rate. This would handicap a cow, which must both minimise its mass to avoid predators – which would lead to a small rumen

size, and maximise its return from low grade food – which would lead to maximum retention times and thus a large rumen. The cow, and other ruminants, resolve this by having a mechanism for sorting particles and allowing only small ones to pass forward to the omasum, and retaining large ones to be ruminated. This ensures that undigested particles (which will tend to be larger) stay in the rumen, but particles which are largely digested are fed forwards.

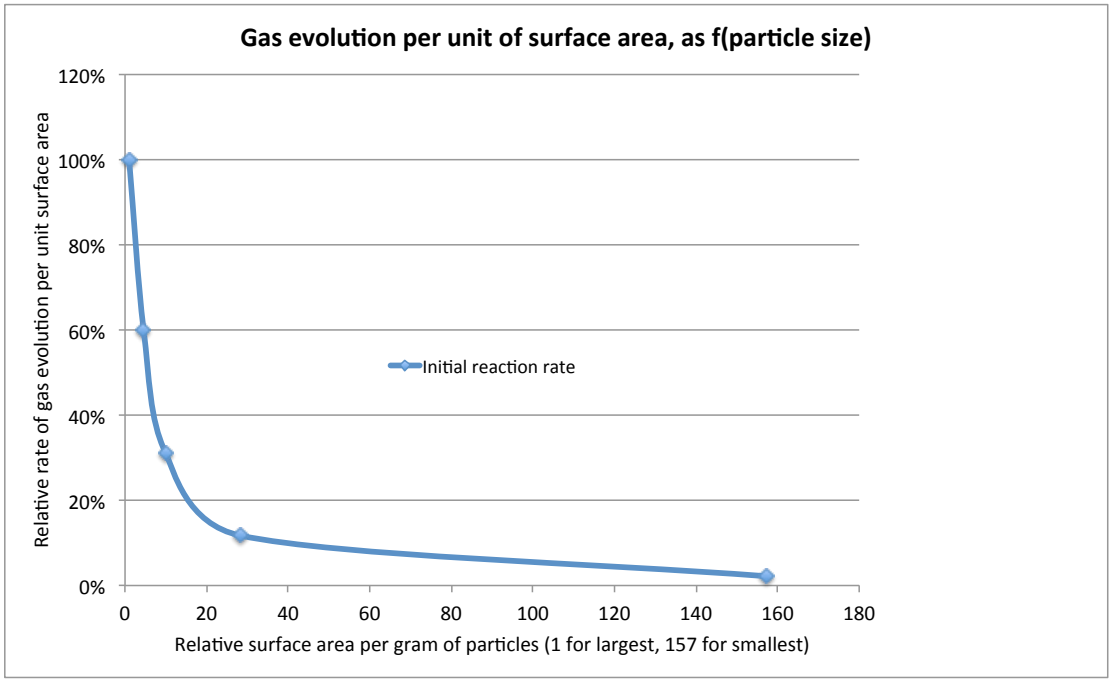


Figure 34 - Re-analysis of Sharma data on digestion rate as a function of particle size, showing the rate of digestion at the start of digestion as a function of the relative surface area of particles at the start of digestion. Surface area calculated as for Figure 33.

The omasum then traps the small particles and may provide a more suitable environment for their digestion. It may also explain why cows do not chew their food well initially, as this would lead to an increased proportion passing directly to out of the rumen.

Kangaroos, on the other hand, have a small sacciform compartment, followed by a relatively large tubiform compartment which behaves like a plug flow reactor. There is no mechanism for sorting particles, and all particles travel through the main, tubiform, part of the digestive system at the same speed.[163] This ensures that all particles are digested for more or less the same time – thus avoiding the short circuiting problem of the CSTR and one possible need for rumination. It does retain small particles longer than may be necessary, but as these shrink with digestion time the efficiency penalty may be minor.

One problem with a tubiform digestive system is that it hinders the inoculation of fresh feed with the requisite microbial biota. In a plug flow reactor there is little axial mixing and both liquid and solids move in the same direction. Thus fresh feed may not be exposed to enough microbial matter to inoculate it and accelerate the necessary biofilm formation. (*This is discussed further in Chapter 10 on modelling biofilms*). One way to avoid this problem is to have a small CSTR like fore-stomach which provides an opportunity for fresh feed to be mixed with already colonised feed, to speed up the process of colonisation and biofilm formation. This is exactly what is seen in kangaroos. Merycism may possibly be a way to enhance this process as and when needed.

It is interesting to note also that mammals that have hindgut fermentation also have the problem kangaroos face with inoculation. Many of these have evolved a 'washback' mechanism to bring microbe rich liquid forwards in a retrograde direction from colon to caecum.[166]

SALIVA

Saliva is seen to be critical to the digestive processes of both ruminants and grazing macropods and the composition of saliva has been investigated by a number of researchers.[161,162,164,167,168]

Beal, in particular, noted the similarity of the chemistry of macropod and ruminant saliva and suggested that this example of convergent evolution demonstrated the importance of this particular saliva chemistry to foregut fermentation.[164]

Figure 35 shows a comparative analysis of the saliva of cows, sheep and red kangaroos. The data for cows comes from Hungate, from sheep from McDougall, from red kangaroos from Beal.[157,162,164] In addition, to compare the saliva composition of a non-grazing omnivorous animal, the composition of human saliva has also been included. Human pH data came from Humphrey, and the remaining data from Veerman.[169,170] In order to show the data on a single graph, all data have been normalised with sheep saliva representing 100%. All the data are expressed as ranges where these are known, as saliva composition can change quite significantly as a function of stimulus and flow rate.

The levels of bicarbonate, phosphate and pH are remarkably similar between the three grazing animals, and completely distinct from those of humans. The levels of these are also quite

different to serum levels in the animals, and so presumably there is some cost to reproduce them.[164] This suggests that these factors are crucial to foregut fermentation of grasses.

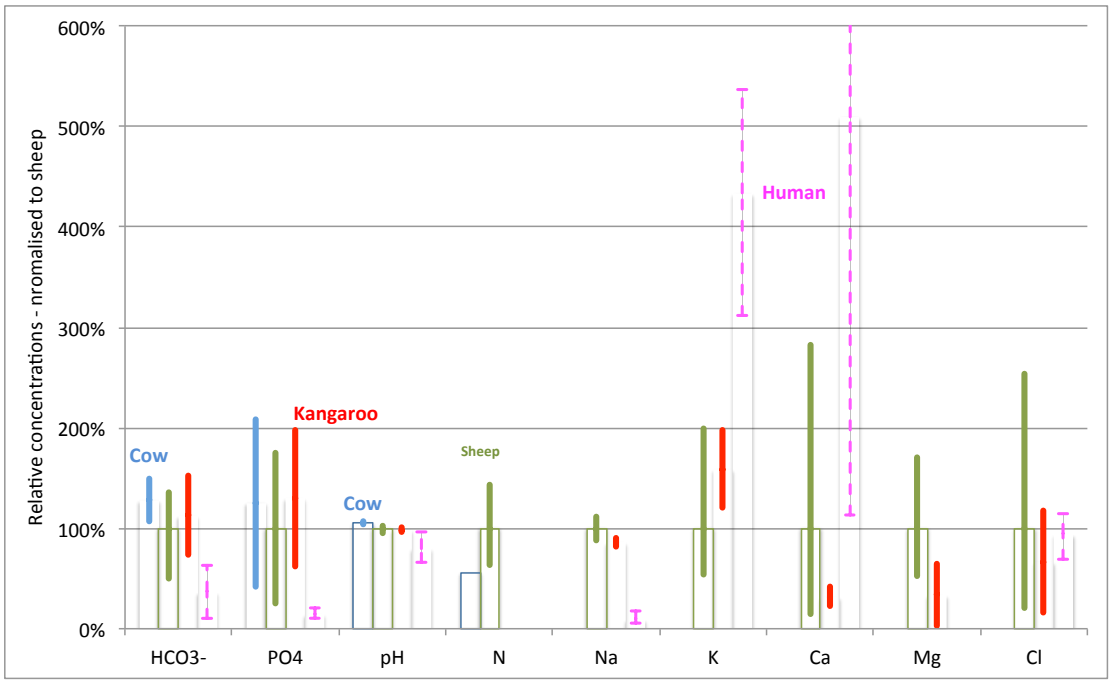


Figure 35 – Comparison of the chemistry of saliva in cows, sheep, kangaroos and humans. Coloured bars represent the reported ranges of the data where available. Human saliva is used as a proxy for non-grazing mammals, and shows considerable differences from the saliva of grazers, whereas grazers as different as cows and kangaroos show remarkable similarities.

Nitrogen, as urea, is quoted as a range for sheep, but as a single value for cows, and not at all for kangaroos. The other components, with the exception of magnesium, show overlap between sheep and kangaroos (there is no data for cows) though the congruence between the species is much less evident than for the first three parameters. Humans, though, are distinctly different.

Ascorbic Acid

Prévot and Taffanel (1946), as reported in Eddy and Ingram (1953), noted that ascorbic acid as an anti-oxidant allows anaerobes to thrive in oxic environments, and also that certain anaerobes show enhanced VFA production of between 2x and 7x in the presence of ascorbic acid at a concentration of 0.1mMol, though it is not clear whether this refers to the rate of production or the total amount produced.[171,172]

Giuliano and Khan examined the effect of various compounds on the activity of endoglucanase produced by *bacterioides cellulolovorans* and on the filter paper activity of the same bacteria.[173]

They observed 100% increase in filter paper activity, and almost 400% increase in endoglucanase activity in the presence of 1mM ascorbic acid.

Reid et al. detected and investigated the levels of ascorbic acid in cow saliva.[161] They measured levels in both saliva and blood in five cows, at four distinct times of the day; midnight, before the morning feed, before the afternoon feed, and after the afternoon feed.

Reid discounts any relationship between ascorbic acid levels in the saliva and the blood, but re-analysis of his data suggests otherwise. Figure 36 shows the levels in blood and in saliva for the 5 cows at each of the four times of day. It is clear that both before the morning feed and after the afternoon feed the two parameters become closely correlated, whereas at other times there is only a weak or non-existent relationship. It is difficult to know what this means, but it does suggest that ascorbic acid has a function which is associated with feeding.

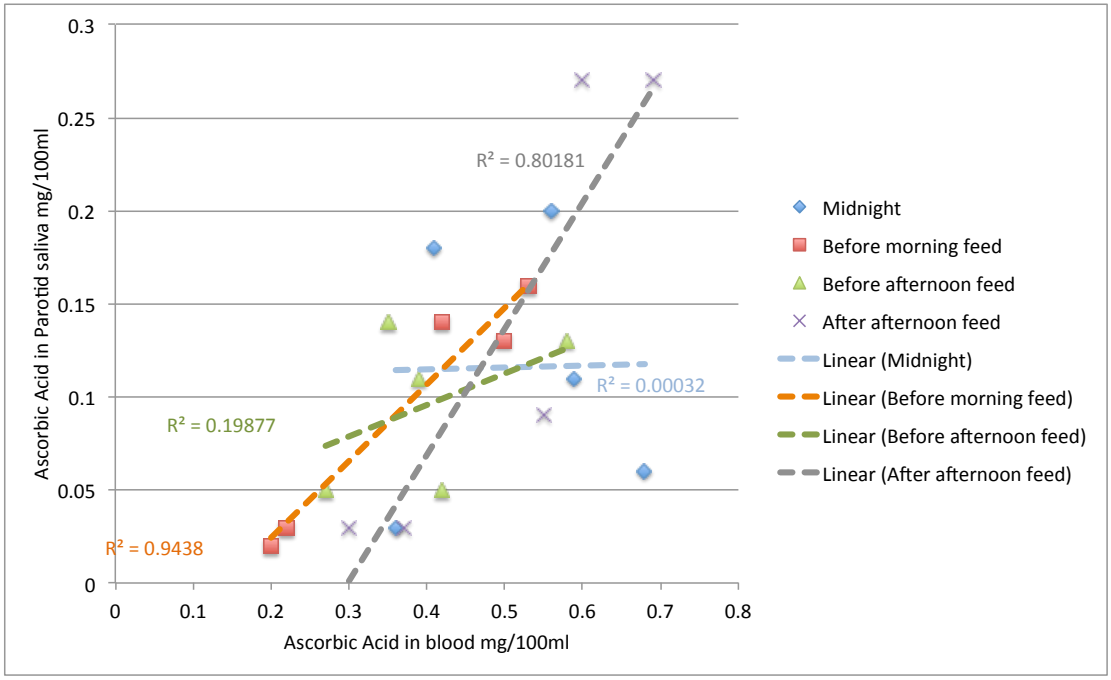


Figure 36 - Ascorbic acid ratios in blood and saliva in relation to feeding. Dotted lines show best linear regression analyses of the ascorbic acid rations for different times of day. It would appear that there is no correlation between these two concentrations at midnight, but before the morning feed, and after the afternoon feed a strong correlation emerges.

The huge variation in these concentrations gives cause for reflection, but the consistency of the message – ascorbic acid enhances cellulolysis and is present in cow saliva – suggests that this is an important area to investigate. The age of these references is therefore a surprise. The

mechanism is unresolved, but Eddy and Ingram note that the reversible oxidation of ascorbic acid to dehydroascorbic acid results in the uptake and release of hydrogen, which may enable it to play a role as an intermediate hydrogen carrier.[172] This may go some way to explaining the activity of ascorbic acid, and its role in saliva.

AD Research using Rumen fluids and Saliva

McDougall went so far as to propose a formula for artificial saliva that might be of use to future biochemists and bacteriologists.[162]

Song examined the effect of using rumen fluid instead of more conventional leachate in batch tests of AD reactors digesting pure cellulose.[174] Two parallel experimental lines were followed – one with 100% rumen based inoculum, and the other with 20% inoculum and 80% artificial saliva as proposed by Coleman.[175] The rumen inoculated experimental reactors rapidly soured as a result of the high rates of hydrolysis which were unmatched by the rates of methanogenesis. Given that a cow is attempting to minimise methanogenesis as a potential loss of energy, the probable shortage of methanogens in rumen fluid means that it is not unreasonable to expect such a result – even in the presence of initial significant buffering capacity. Nonetheless, the rumen fluid reactors did exhibit rates of hydrolysis prior to souring of 2x-3x those from reactors inoculated by conventional means.

O'Sullivan carried out similar batch experiments using a different approach to buffering, and demonstrated very significant differences between reactors inoculated with landfill leachate and those inoculated with rumen fluid.[176] In O'Sullivan's experiments the leachate inoculated reactors achieved 80-85% digestion in 12-30 days, whereas the rumen inoculated reactors achieved 95% solubilisation after only 7 days.

O'Sullivan's rates of hydrolysis were comparable to those from other investigators, and in particular the work of Gijzen and subsequent experimenters.[177–179] This work confirmed the importance of using rumen based inoculum, and having sufficient buffering to allow the rapid acidification of the substrate. Gijzen also demonstrated that recirculating the liquid from the acidogenic reactor through a methanogenic reactor and back to the acidogenic reactor had the effect of transferring methanogens to the acidogenic reactor. These then converted most of the

acetic and butyric acids to methane in situ – so that the methanogenic reactor was left more or less solely as a propionate degrader. This also had the effect of raising the pH of the acidogenic reactor sufficiently to avoid acid inhibition.

None of the research appears yet to have identified what it is about rumen fluid that makes it more vigorous than reactor inoculum, and none has established that in the long term the vigour will not decay to that of standard reactor inoculum. This would therefore seem to be the key area for future research.

8.4 THE ROLE OF PROTOZOA AND FUNGI

Many protozoa are cellulolytic, though unlike bacteria which hydrolyse externally, the protozoa do so internally.[180] Whilst protozoa are much less numerous in the rumen of cattle and sheep than are bacteria, their contribution to the digestive process is significant – in part because of their large size compared to bacteria. Thus up to 40% of rumen nitrogen in cattle on a high grain ration may be found in the protozoa; and 30%-60% of the fermentation products in a rumen may be protozoal.[181,182]

There has been considerable speculation regarding the importance of protozoa to ruminant digestion. Numerous early authors as cited in Hungate evaluated the effect of de-faunation of ruminants and subsequent re-faunation with no protozoa, and observed little or no effect on growth.[183] More recent work by Luther et al. demonstrated a modest effect on VFA production in a comparison of lambs with or without protozoa.[184] The presence of protozoa increased ruminal VFA concentrations by around 20% provided the lambs were fed a high roughage diet. Lambs fed a high concentrate diet showed no significant differences. Orpin examined the activity of ciliate protozoa *in vitro* and observed that they were able to colonise rapidly (within 15 minutes) the damaged ends of plant fibres. Attachment at the oral cavity suggests they were involved in digestion.[185]

It is difficult, from the literature, to get any clear idea of the function of protozoa in the rumen. They are clearly important but equally they are not essential, though there may be some

circumstances where they moderate the effects of a high cellulose diet by increasing the efficiency of cellulolysis.

Fungi are also present in the rumen where they may have a significant role in lignin degradation and thus increasing the digestibility of the residual biomass by bacteria and protozoa. Orpin determined that a pure cultures of *Neocallimastrix frontalis, Piromonas communis* and *Sphaeromonas communis*, could solubilise up to 22% of the lignin in wheat straw and hay, but that none of the lignin appeared to be used as a carbon source by the fungi.[185] This conclusion is supported by the work of others and leads to the view that a key role of fungi may be to increase the digestibility of difficult substrates rather than to accelerate the rate of digestion.[186–188]

The previous studies were all *in vitro*. Gordon and Phillips examined the effect of the removal of fungi from the rumens of sheep *in vivo*, by running first a control, then removing all fungi, and then re-inoculating the sheep with fungi.[189] The sheep with fungi ate 40% more of a straw based diet than did the same sheep with no fungi. This reinforces the view that fungi have an important role in increasing the availability of digestible material, and do so fast enough to improve the digestion of living ruminants.

Chapter 9 BIOFILMS, BUBBLES AND BOUNDARY LAYERS

There is a general presumption in the literature that the important parameters governing AD can be gleaned by measuring the bulk parameters of the menstruum in a reactor. This chapter explores the extent to which this may be false and misleading. It starts from the perspective of the bacterium, which is an almost immobile creature in contact only with the thin veneer of molecules that surround it. This boundary layer, and its significance for reaction kinetics, is both an issue for bacteria – within or outside a biofilm, and also small particles of substrate that carry the biofilms. The only ways to change the contents of this boundary layer are by diffusion or disruption which allows advection. The following sections discuss what the properties of these biofilms and the boundary layers might be and how they might affect the processes underlying AD.

9.1 ADVECTION, DIFFUSION AND BOUNDARY LAYERS

The Reynolds Number, (Re) is the dimensionless parameter that describes the ratio of inertial forces to viscosity forces experienced by a particle in a fluid flow. Key components of Re are the dimensions of the system and the relative velocity of the particle in the liquid flow. When Re is high, the flow around the particle is turbulent, and eddies connect the surface of the particle to the bulk of the fluid. Turbulent flow enables rapid mass transfer between a particle and the medium. Conversely, when Re is low, the flow around the particle is laminar, and streamlines remain parallel. In this flow environment, diffusion is the dominant mass transfer process, which is much less effective than eddies.

As a general rule, Re > 4 000 is associated with turbulent flow, whereas Re < 2 000 is associated with laminar flow. Re is calculated as follows:

$$Re = \frac{uL}{v} \tag{1}$$

where u is the relative velocity of particle and fluid, L is the characteristic length, and v is the kinematic viscosity. The relative velocity is the most difficult parameter to determine as it, in turn, is dependent on the forces it feels, and whether the flow regime is turbulent or laminar.

The relative velocity in steady flow can be expressed in terms of the force it faces, using Stokes Law provided the flow is laminar:

$$v = \frac{2}{9}\frac{(\rho_p - \rho_f)}{\mu} a \frac{L^2}{4} \quad (2)$$

where ρ represents the densities of the particle and fluid respectively, μ is the dynamic viscosity, a is the acceleration, and L is the diameter of the particle. The density of the particles is somewhat difficult to determine, as it varies across the course of digestion as a function largely of entrained air at feeding time, and then the formation of adherent bubbles. This manifests in particles alternately sinking and floating. At all times though it is close to that of water. For the purposes of this analysis a 5% difference between the density of feedstock and the density of water has been assumed.

Substituting equation 2 in 1 allows the construction of a plot showing Re against acceleration measured as the number of 'g' the particle faces for a range of particle sizes (Figure 37). This shows that for a perfect sphere, even particles of 10mm diameter at 10g are in a laminar flow environment.

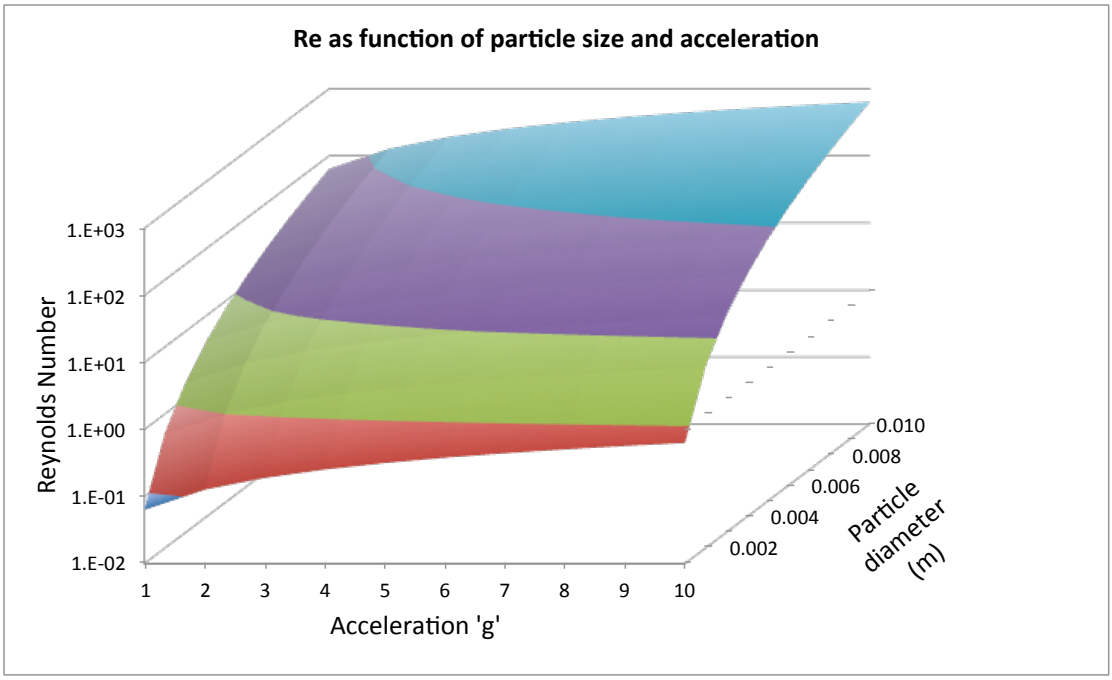


Figure 37 - Reynolds number as a function of acceleration (measured as number of 'g') and particle sizes from 1cm to 1mm. Using Re<2 000 as a cutoff for laminar flow, the graph shows that even particles as large as 10mm under accelerations of 10g experience laminar rather than turbulent flow.

Having established that most particles are exposed to laminar flow, it is appropriate then to use Stokes Law to derive an approximation of the relative velocity of particle and fluid.

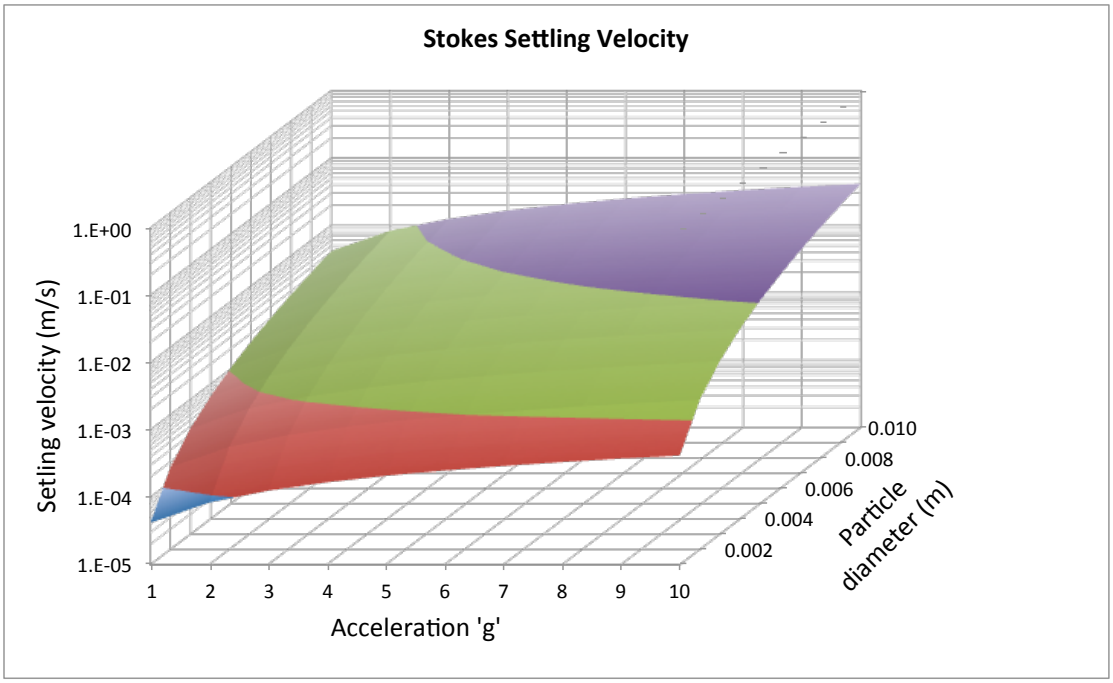


Figure 38 - Settling velocity of a spherical particle under steady state in water as a function of particle size and acceleration.

Figure 38 demonstrates that for perfect small particles, of the order of 2mm in diameter, settling velocities other than under extreme acceleration are of the order of 0.1-1.0mm/sec. These equations are designed for an ideal situation, namely a sphere in a steady state flow in water. The key simplifications have the following effects:

1. In the real world the flow will be locally unsteady in any stirred tank. To understand the motion of a particle in these circumstances requires solution of the Basset-Boussineq-Oseen equation. This introduces a number of other terms beyond the Stokes drag, the most significant of which is the 'added mass' of the particle. This represents the mass of fluid the particle is dragging with it in its boundary layer. In a liquid of comparable density to the particle this added mass is significant, and increases the mass that has to be accelerated by the available force. The force that causes the particle to have a different velocity to the fluid arises from the different densities of the particle and the fluid – and is unaffected by the added mass. Thus the same force is applied to a larger

mass – which leads to the particle effectively tracking the fluid velocity more closely than if it is ignored – thus reducing the relative flow between particle and fluid.

2. The particles are far from spherical. This will tend to increase drag and reduce the settling velocity, and thus increase the relative effects of boundary layers
3. The viscosity is not that of water. Many reactors, in pursuit of smaller particle sizes, comminute their feedstock. In other reactors, the reduction in particle size that comes about naturally as a result of digestion also leads to a colloidal solution forming. Higher viscosity will lead to significantly lower relative velocities.

The foregoing analysis supports the proposition that biomass particles in a CSTR will be exposed to laminar flow and very low settling velocities unless exceedingly high 'g' forces are created which would have extreme energy costs. It is appropriate therefore to consider what the boundary layer conditions and diffusion limitations might imply for the speed of hydrolysis.

BOUNDARY LAYERS AND DIFFUSION

In a laminar flow environment diffusion is the only method of transporting material from the surface of a particle of biomass into the bulk medium, and the effective distance that material has to diffuse before being fully diluted by the bulk medium is a function of the thickness of the boundary layer, and the relative velocity of particle and fluid.

At the surface of a particle moving in a fluid stream, the fluid velocity relative to the particle will be zero. As the distance increases from the particle, so the velocity of the fluid will increasingly approach the stream velocity. This velocity transition zone is known as the boundary layer across which diffusion has to take place. For particles with a high relative velocity compared to the bulk medium, the boundary layer effects are diminished because the diffusing substance soon enters a zone of higher velocity fluid and gets moved away and the 'diffusion barrier' may be seen to be low. However in low velocity environments, such as small biomass particles in a viscous fluid of similar density to the particle, the 'diffusion barrier' becomes comparatively large.

An informative discussion of boundary layer effects in a closely related area – marine micro-organisms, can be found in a paper by Stocker.[190] Figure 39 shows how long it takes for a single pulse of chemicals in the boundary layer to dissipate in still water.

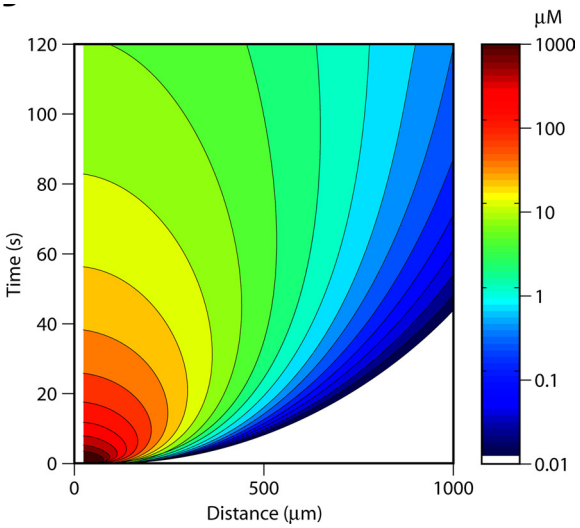


Figure 39 - Illustration of changing concentration over time and distance of a nutrient pulse into a micro-organism boundary layer. The data reflect a lysis event of a cell 25 µ radius, an internal concentration of 100mM, and a diffusion coefficient of the solute of 7.2 x 10-10 m2/sec. (*Image from Stocker*).

Lens and Beer examined both pH and glucose concentration gradients outside and within methanogenic granules from UASB reactor.[191] The granules were around 1.5mm diameter. They determined a diffusive boundary layer around a granule of 100-200µm for glucose and pH when the relative flow velocity between the particle and menstruum was around 0.5mm/s. This layer was seen to act as providing significant resistance to mass transfer.

A planktonic bacterium is much smaller than a particle of feedstock. Its small size and high water content mean that it will experience almost no settling, and the diffusive barrier will be high. Bacteria in a biofilm will benefit from the settling velocity of the particle to which they are attached, but the effective distance for diffusion may be increased as the channels linking the interior to the surface of the film may be far from straight.

This suggests that the properties of the menstruum, that are easy to measure in practical operations of AD (e.g. pH, Ripley Ratio), are only indirectly related to the environment actually experienced by the bacteria.

9.2 Development of Biofilms

The analysis above largely considers the perspective of an individual bacterium, but this is not how most cellulolytic bacteria operate. Biofilms are the normal form of existence for 99% of

bacteria, and an understanding of anaerobic cellulolytic biofilms is essential to understanding the efficacy of ruminant and macropod digestive systems, and perhaps the inefficiency of commercial AD.[192]

Cellulose is insoluble, and bacteria digest it by expressing enzymes that convert it to simple sugars. As discussed previously in Chapter 7, the high investment cost of producing an enzyme makes it unlikely that a bacterium in its planktonic form can afford to express enzymes and release them into the bulk medium of a digester in the hope of securing a food supply. The greatest chance of success for a cellulolyte comes from firmly attaching itself to a substrate surface, and expressing its enzymes close to itself – or even keeping the enzyme attached in the form of a cellulosome. This assumption is supported by a number of authors, who observe that the bulk of endoglucanase and xylanase activity is by substrate bound organisms.[131,193,194] There will of course be some bacteria that are free in the liquid, because inevitably, as substrate is consumed the available surface area will decrease and bacteria will become detached.

Once a bacterium is in a position to reproduce, its daughters are unlikely to drift off into the menstruum. This would mean less certainty in finding food. A more likely course, with probable survival benefits, would be for a daughter to attach immediately adjacent to the parent if they are able – thus eventually forming a biofilm.[192]

This leads to the conclusions that areas of lignocellulose that are not covered with biofilm cannot be digested by bacteria, i.e. all hydrolysis of lignocellulose occurs within these biofilms – and that no significant hydrolysing bacteria are active in the menstruum. This is supported by studies that show minimal bacterial activity in the liquid portion of a reactor and that the rate of solubilisation is directly dependent on the surface area covered by bacteria.[134] O'Sullivan also demonstrated (Figure 23) that biofilms on particles of pure cellulose took up to 41 days to fully cover the surface, with maximum rates of solubilisation occurring between days 3 and 18. This is substantially longer than the residence time of solids in the rumen, even before the complexities of digestion of plant materials – with their defences and lignin, are taken into account.

ADHESION

The formation of biofilms requires adhesion of the bacteria to the surface. There have been a number of attempts to build physico-chemical theories of adhesion, but the complexity of the process, and the multiplicity of species involved means that these cannot explain many aspects of the process, or predict outcomes.[195]

The predominant ruminal cellulolytic bacteria have limited ability to move independently [130], and thus little ability to actively penetrate the electrical fields, fluid boundary layers and surface forces that surround particles of substrate. They are therefore highly dependent on being presented with the right physical, chemical and electrical environment for attachment. Surface tension is one key factor whose importance has been quantified albeit for other bacteria than cellulolytes, and on manufactured polymeric surfaces.[196] Likewise charge density and shape have been shown to be important, with small radii on the substrate or the bacterium, or surface scratching, being important factors.[197–199]

Once they have arrived at a surface bacteria may need to overcome any resistance from the plant cells to bacterial infection. Plants have evolved over the aeons to resist infection by bacteria, and it seems likely that some elements of this resistance persist after the plant death. In addition, many tissue surfaces are recalcitrant.[200] This means that bacterial cells may take a considerable time to attach, and that attachment and infection are important elements in the overall speed of hydrolysis.

Certain chemical and physical properties of the system may be crucial in promoting adhesion. Monds and O'Toole determined the critical role of phosphates in adhesion.[201] A further factor that has been observed is the relationship between pH and adhesion, with lower adhesion rates at low pH than at higher pH.[202]

9.3 HETEROGENEITY IN BIOFILMS

Within a biofilm no degree of stirring of the menstruum will be effective in homogenising the fluids, and diffusion becomes the limiting phenomenon.[203] As a result transport of nutrients and waste products around the film will be dependent either on recycling by other organisms or by

somewhat restricted diffusion. The diffusion from inside to outside the biofilm will be further restricted by diffusion through the particle boundary layer as previously discussed. This will lead to both heterogeneity of chemical properties within the biofilm, and a disconnection between the average properties of the biofilm and the menstruum.

A useful discussion of diffusion through biofilms is to be found in a paper by Stewart.[203] Stewart demonstrates the times required for various chemicals, with diffusivity constants similar to the VFAs, to penetrate biofilms of thicknesses ranging from 100-1000μ to range from 165s to 714s. The key observation is that the time taken to diffuse is proportional to the square of the diffusion distance. Thus diffusing through a film 10 cells deep will take 100x as long as diffusing across a single cell, even ignoring any increase in path length associated with the actual travel distance from the interior of a film to the exterior. This will undoubtedly result in high concentration gradients across a biofilm, which will be compounded further by the effective depth of the boundary layer.

One particular issue that heterogeneity affects is VFA and pH inhibition of cellulolysis. Cellulolytes express VFAs as a waste product, which are inhibitory. One explanation for the inhibition is that, if the pH outside a cellulolyte is lower than the intracellular pH, the concentration of undissociated and liposoluble VFAs outside will be greater than inside, and there will be a tendency for diffusion inwards through the cell walls. Once inside, in a higher pH environment, dissociation will impede outward migration.[148,151,154,202,204,205]

Lema et al. modelled the transport associated Gibbs energy production rates for a hypothetical glycolytic bacterium transporting 1 molecule of lactate across the cell wall for each glucose molecule consumed, at a range of pH values.[206] They calculated that at a pH >7.5, diffusive transport out of the cell added to the available Gibbs energy, and below 7.5 reduced it. However below pH 5.5 the rate of loss of energy increased dramatically, so that at pH 4.5 approximately 80% of the Gibbs energy from glycolysis is consumed in lactate transport (Figure 40).

The limitations of diffusion within biofilms means that the concentration of undissociated VFAs in the immediate vicinity of a cell may be significantly higher than in the menstruum unless there is either an effective removal method or a means to keep the pH high. As an example of this

heterogeneity, Lens and Beer examined the change in pH with depth in methanogenic aggregates and demonstrated that fresh granules showed a >1.5 pH unit decrease in around 600 μm from the surface when exposed to glucose at reactor concentrations.[191]

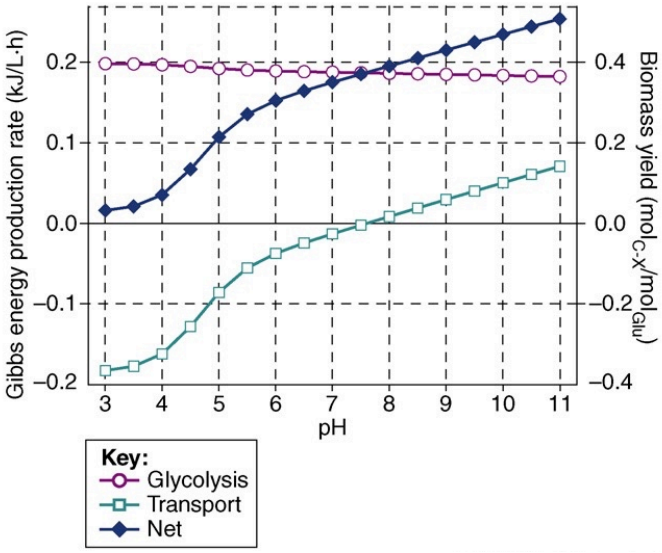


Figure 40 - Gibbs energy production and biomass growth as a function of pH for the bacterial conversion of glucose to lactate. Below pH of 7.5 the bacterium has to use energy to extrude lactate against the pH gradient, with a resultant loss in both free energy and biomass growth. (*Graph copied from Lema et al. 2008*)

Similarly in dental plaque (a biofilm) in humans the pH within the plaque can be as low as 5.5 whereas the pH of the mouth is broadly neutral.[205] A more extreme example of heterogeneity comes from investigation of natural biofilms using solid state micro-electrodes. This has shown that points in a biofilm only microns apart may have pH differences of up to 7 units (*sic*).[207]

Veeken and Kalyuzhnyi examined the effects of pH and VFA concentration on the solubilisation of lignocellulose, and demonstrated that at high VFA concentrations low pH had a significant inhibitory effect, though they only considered pH levels down to 5.[153]

Bubbles and Supersaturated Solutions

In addition to heterogeneity being created by limited diffusion, an additional problem may arise as a result of cellulolytic bacteria that produce gaseous wastes that are normally, at atmospheric conditions, only slightly water soluble. In the context of AD, these are principally CO_2 and H_2.

Assuming, for the present, that the CO_2 and H_2 form bubbles on the surface of the bacterium, then the excess pressure (P) within the bubbles can be calculated as:

$$P = \frac{2T}{r} \quad (3)$$

where T is the surface tension, and r is the bubble radius.

Assume the bubble is of a similar diameter as the bacterium – namely 2 μm ($r = 10^{-6}$m) and the surface tension of water (T) is 70mN/m at 37 °C then the excess pressure is as follows:

$$P = 2 \times 70 \times 10^{-3} / 10^{-6} = 1.4 \times 10^{5} \text{ Pa.} \quad (4)$$

In addition the atmospheric pressure plus the pressure depth of the bubble in the reactor need to be added. Assume atmospheric pressure is 10^5 Pa , and the bubble is 5m below the surface of a reactor – adding a further 5×10^4 Pa of pressure – then the pressure in the bubble will be 2.9×10^5 Pa. If the bubble is comprised of equal molar quantities of CO_2 and H_2 then the partial pressure of CO_2 (pCO_2) will be 1.45×10^5 Pa, or approximately 1.4 atmospheres (atm.).

Figure 41 shows a graph of the partial pressure of CO_2 in a gas bubble of 50% CO_2 as a function of diameter, assuming the bubble is 5m below the surface of a commercial scale AD reactor.

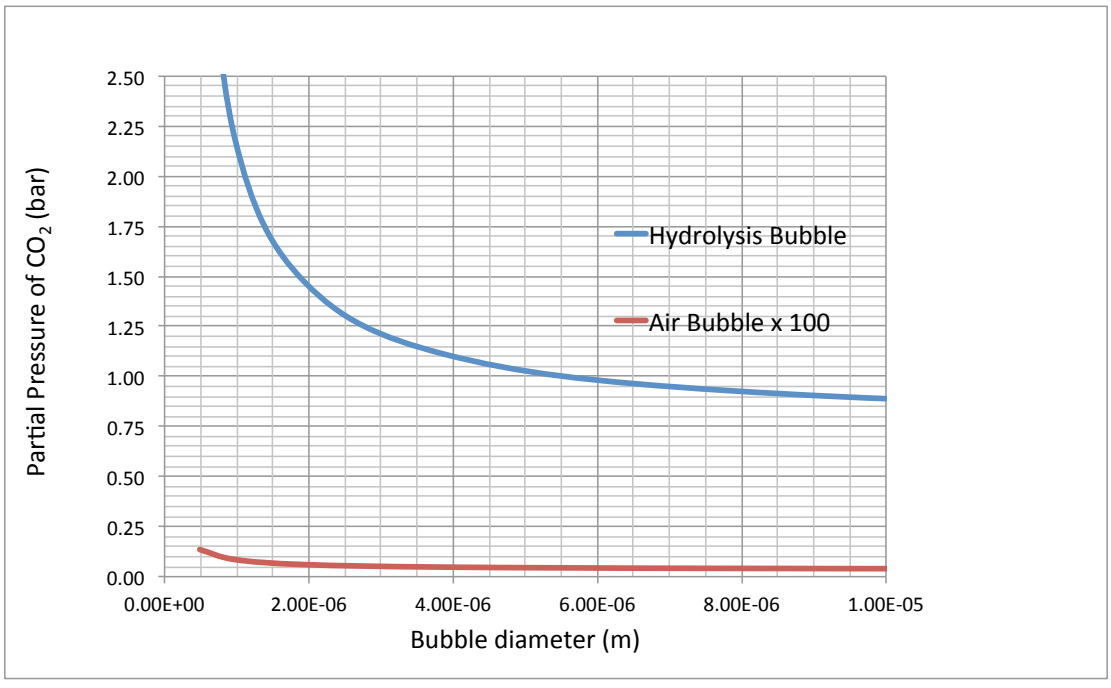


Figure 41 - Partial pressure of CO_2 in a bubble of hydrolysis gas at 5m below the surface of an AD reactor, compared to an identical bubble of air. Note the partial pressure of CO_2 in a bubble of air is shown at 100x the actual pressure in order to be visible on the graph.

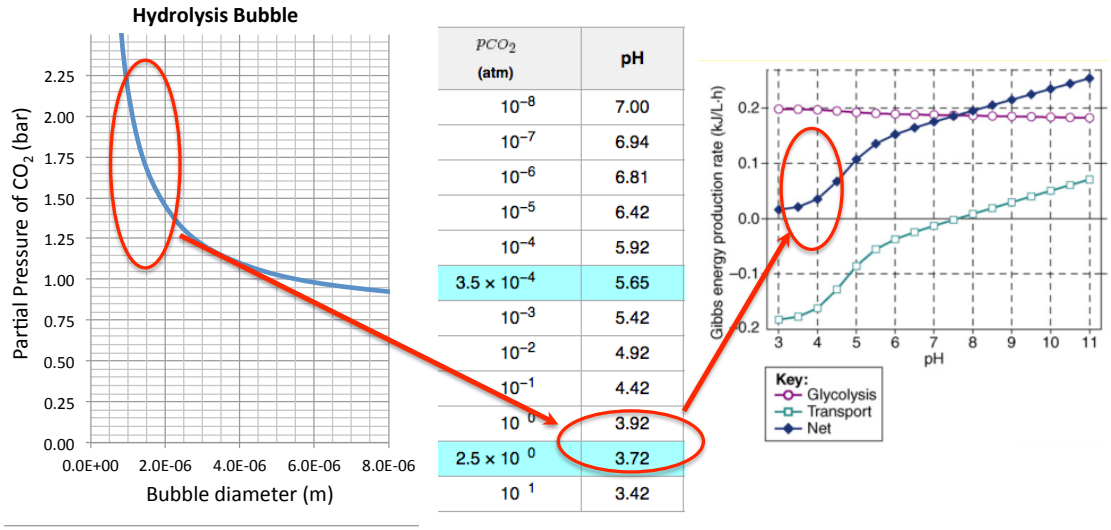
Table 13 shows the pH of water exposed to CO_2 at a range of partial pressures. For reference the pH of water in equilibrium with air at a pCO_2 of 3.5 x 10^{-4} atm. is 5.65 and of carbonated soda water, at pCO_2 of around 2.5 atm., is about 3.7. At the partial pressure of 1.4 bar, as predicted for this hypothetical bubble, the pH of pure water would be around 3.8.

Table 13 - pH of water at various values of pCO_2 (extracted from Wikepedia).[208] The upper blue band shows the equilibrium pH of water in contact with air, resulting from the partial pressure of atmospheric CO_2. The lower blue band shows, for reference, the pH of carbonated soda water.

PCO_2 (atm)	pH	$[CO_2]$ (mol/L)	$[H_2CO_3]$ (mol/L)	$[HCO_3^-]$ (mol/L)	$[CO_3^{2-}]$ (mol/L)
10^{-8}	7.00	3.36×10^{-10}	5.71×10^{-13}	1.42×10^{-9}	7.90×10^{-13}
10^{-7}	6.94	3.36×10^{-9}	5.71×10^{-12}	5.90×10^{-9}	1.90×10^{-12}
10^{-6}	6.81	3.36×10^{-8}	5.71×10^{-11}	9.16×10^{-8}	3.30×10^{-11}
10^{-5}	6.42	3.36×10^{-7}	5.71×10^{-10}	3.78×10^{-7}	4.53×10^{-11}
10^{-4}	5.92	3.36×10^{-6}	5.71×10^{-9}	1.19×10^{-6}	5.57×10^{-11}
3.5×10^{-4}	5.65	1.18×10^{-5}	2.00×10^{-8}	2.23×10^{-6}	5.60×10^{-11}
10^{-3}	5.42	3.36×10^{-5}	5.71×10^{-8}	3.78×10^{-6}	5.61×10^{-11}
10^{-2}	4.92	3.36×10^{-4}	5.71×10^{-7}	1.19×10^{-5}	5.61×10^{-11}
10^{-1}	4.42	3.36×10^{-3}	5.71×10^{-6}	3.78×10^{-5}	5.61×10^{-11}
10^{0}	3.92	3.36×10^{-2}	5.71×10^{-5}	1.20×10^{-4}	5.61×10^{-11}
2.5×10^{0}	3.72	8.40×10^{-2}	1.43×10^{-4}	1.89×10^{-4}	5.61×10^{-11}
10^{1}	3.42	3.36×10^{-1}	5.71×10^{-4}	3.78×10^{-4}	5.61×10^{-11}

Using these calculations and this data Figure 42 relates the partial pressures in bubbles of gas from glycolysis to the energy available to bacteria from glycolysis in a reactor at 5m below the surface. The analysis suggests that the formation of micro-bubbles in a biofilm of cellulolytic bacteria may result in highly localised areas of very low pH within the boundary layer of the cellulolyte. Unless there is an effective mechanism for removing the CO_2 this could create strongly inhibitory conditions with the result that the upper limit for the rate of hydrolysis becomes in effect controlled by the rate of boundary layer diffusion.

An additional factor is the gaseous nature of H_2 and CO_2. These gases have to find their way out of the film, and in doing so will occupy a substantial proportion of the void volumes that might exist. This will add to the problem of diffusion of the liquid phase inhibitors. Thus, within the diffusive barrier, CO_2 compounds the inhibitory tendencies of VFAs to form an environment in which bacteria have to expend the vast proportion of their energy in VFA transport against a severe pH and VFA concentration gradient.



PCO_2 (atm)	pH
10^{-8}	7.00
10^{-7}	6.94
10^{-6}	6.81
10^{-5}	6.42
10^{-4}	5.92
3.5×10^{-4}	5.65
10^{-3}	5.42
10^{-2}	4.92
10^{-1}	4.42
10^{0}	3.92
2.5×10^{0}	3.72
10^{1}	3.42

Figure 42 - Relationship between partial pressure of CO2 from glycolysis 5M below the surface of a commercial reactor and Gibbs energy available to a bacterium. The red line on the left hand graph shows pCO2 for a bubble of atmosphere under the same conditions for comparison.

Paradoxically this pH/VFA inhibition may in fact favour VFA production. Production of enzymes and bacterial growth are both energy and mass consuming activities. If harvesting and retaining energy and carbon from glycolysis is too efficient, the circumstances may favour bacterial growth over VFA production. Conversely, high energy requirements for VFA transport in a low pH environment may require the bacterium to use glucose to produce more VFA and devote less of the mass and energy to bacterial growth and enzyme production. Thus low pH within the biofilm may be beneficial to both ruminant and anaerobic digestion by promoting VFA production over bacterial growth. However ultimately the rate of VFA and CO_2 production may well overwhelm the rate of removal, and drive the pH so low that either bacterial activity becomes energetically impossible or enzyme production is severely inhibited. In these circumstances the rate of glycolysis would be expected to come into equilibrium with the rate of CO_2 and VFA removal. The combination of the problems of diffusion and boundary layers, coupled with extreme heterogeneity within the biofilms may offer a possible explanation of the brake on the system illustrated by the Sharma data.

Chapter 10 COMMUNITY GROWTH – MODELLING

"All models are wrong, but some are useful" – George Box, statistician.[209]

A number of investigators have sought to use Monod type reaction kinetics to describe the processes relevant to anaerobic digestion.[206] Such models are descriptive, and offer no insights into why the rates of reaction take the values that they do, or how they can be manipulated to mimic the efficacy of ruminants.

The seeds of an alternative approach can be seen in Conway's mathematical game of "Life".[210] This used a very simple set of rules to govern the behaviour of checkers on a 2 dimensional computerised grid to create an infinite range of self-organising patterns and structures, which illustrated many of the principles of evolution of self-organising systems. The initial modelling work has since been used in a vast range of theoretical papers describing both natural and anthropogenic systems organisation and development. A Google Scholar search of "Game of Life" returns almost 3 million results.

Based on these ideas, and in order to help visualise some of the variability in rate of cellulolysis as a result of adhesion, heterogeneity, bacterial aging and grazing, a grid based model has been built using Matlab to simulate the growth of biofilms on a 3D lignocellulosic substrate. The model makes no attempt to parameterise the input variables, nor to make quantitative predictions. Its purpose is qualitative – to illustrate the nature of some of the processes involved in biofilm growth and how they might interact to create limitations on the process of hydrolysis. However the rules make the model obey certain key constraints; mass is conserved, liquids diffuse from areas of high to areas of low concentration, cells reproduce and die according to the availability of food, local concentrations of waste products and inhibitors and their age; and two forms of cell are recognised, planktonic in a menstruum and sessile on or within the lignocellulose matrix.

10.1 Model Overview

The pseudo-code for the model is contained in Annex 1 together with the actual code. A summary of the main model characteristics is described below.

1. The model runs in discrete time steps.
2. The substrate for the model is a gridded 3D sheet of lignocellulose. Dimensions are specified as unit cells; lignin is randomly distributed throughout the substrate in a variable proportion specified and with strand length also specified.
3. The menstruum outside the boundary layer for the particle is not modelled explicitly, it is defined by a solid:liquid ratio.
4. Bacteria are initially seeded into the model in the menstruum.
    a. Each time step, each bacterium in the menstruum has a probability of finding itself at the solid:liquid interface. The probability is set by the solid:liquid ratio.
    b. Any bacterium at the solid:liquid interface has a probability (an input variable) of settling and becoming sessile.
    c. A bacterium that settles on lignin is inactivated.
5. Each bacterium has properties as follows:
    a. its age, defined by the number of daughters it has produced
    b. the amount of energy it has stored
    c. its location if attached to the substrate.
6. Each time step, sessile bacteria consume a proportion of the available cellulose at the location where they are settled, and produce a quantum of generic inhibitor in proportion to the cellulose consumed.
    a. The amount of cellulose consumed is dependent on the activity level of the bacterium. That in turn is degraded by the age of the bacterium and the level of inhibitor at the bacterial location. The degradation functions for these are specified variable inputs to the model.
    b. The energy stored in the bacterium is incremented in proportion to the amount of cellulose digested.

c. If the stored energy in the bacterium exceeds a specified threshold it produces a daughter bacterium, which can settle either in the same location or in any of the 26 surrounding locations with equal probability.
d. Any bacterium that settles on lignin becomes inactivated.
e. A bacterium that produces more than a pre-determined number of daughters dies. The number is a model input variable.

7. In any time step, if a bacterium is in a cell with no cellulose left and with no cellulose in cells above it , it may detach. The probability of detachment is an input variable.
8. Each time step may be divided into one or more sub-steps in order to model diffusion of the inhibitor. Diffusion is modelled by assuming that molecules of inhibitor cross from one model cell into the adjacent cell in direct proportion to the concentration in the source cell. This is balanced by a diffusion in the opposite direction, again in proportion to concentration, leading to a net flow. The proportion that diffuses is modulated by the diffusivity of the inhibitor, a model input parameter.
    a. At each sub-step, for each face of the cell being modelled an equal proportion, defined by the diffusivity parameter, of the inhibitor contained in the cell crosses the boundary into the adjacent cell. If the adjacent cell is lignin no proportion passes across.
    b. Diffusion also occurs across the solid liquid interface into the boundary layer.
    c. The thickness of the boundary layer is a model input parameter. Beyond the boundary layer the concentration of inhibitor is maintained at constant background level. The level is a variable input parameter.

10.2 INHIBITION IN THE MODEL

The existence of some form of speed limit in AD suggests that at some point in the process inhibition of cellulolytic activity must occur. One objective of the model is to explore what inhibition might look like in practical terms, and how different degrees of inhibition might affect the rate of reaction. At least three modes of inhibition appear to possibly co-exist in a bio-film.

The first, discussed in Chapter 7, relates to the inhibition of enzymatic activity by the sugars created by that activity itself. This inhibition is beyond the scope of the current model to reflect, and it is presumed for modelling purposes that the production, inhibition and consumption of sugars reaches some sort of equilibrium that may be depicted by a single constant reflected by the speed of digestion of cellulose by an otherwise uninhibited bacterium.

The second mode of possible inhibition of cellulolytic activity is a function of the local level of inhibitors such as VFAs which are expressed by the cellulolytes, and as described by various authors in the AD literature.[116,148,151,153,178,211] This is modelled in the software by the use of a function that decreases the relative hydrolytic activity of a bacterium in relation to the local concentration of inhibitor (*INHIBIT in the model*). The default function that defines bacterial activity (BA) before accounting for reduction due to age and the number of daughters is

$$BA = Max(0{,}1 - \log(1 + Inhibitor\ Concentration) * INSENS)$$

The sensitivity of the bacterium to its local concentration of inhibitor (*INSENS in the model*) is an input variable. Figure 43 shows graphically the effect on cellular activity of changing the inhibition sensitivity.

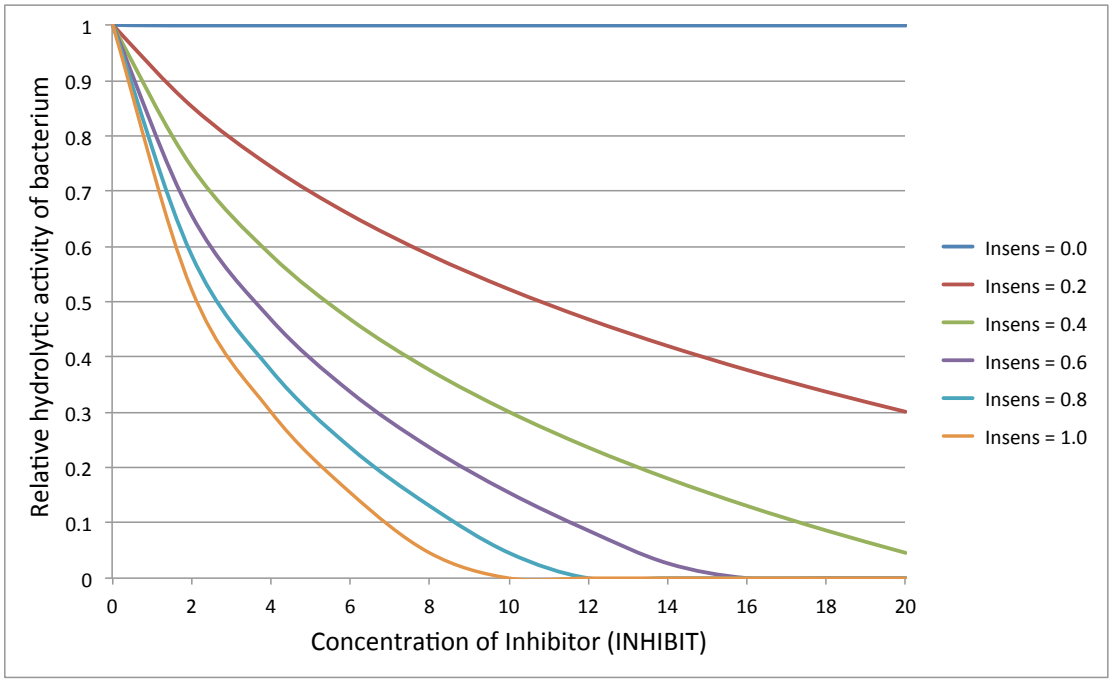


Figure 43 - Graph showing the effect on bacterial activity of changing the sensitivity of the model to inhibition

The third mode of cellulolyte inhibition is that described in Chapter 9 relating to the transport efficiency of VFAs out of the bacterial cell as a function of local pH. This mode of inhibition is not described in the AD literature, and was not foreseen when the model was first constructed and run. A second version of the model (V20a as opposed to V20) was constructed with a modification to illustrate the possible scope of such a mechanism. Under the original model, each unit of cellulose combined resulted in one unit of energy stored in the bacterium, which ultimately resulted in a daughter being produced when the accumulated energy stored reached a critical threshold. Under the revised version, only a proportion of the energy released by digestion of a unit of cellulose is retained; the proportion retained being determined by the local concentration of inhibitor. The function used to determine the relationship was:

$$E_t = E_{t-1} + AA/\max(1, I - 1)$$

where:

E_t = Energy stored in the bacterium at time step t

AA = Units of cellulose digestion activity needed to generate 1 unit of energy E

I = Local level of inhibitor.

This generates a level of stored energy as a function of inhibitor of the form shown in Figure 44. This is a highly schematic representation of that portion of the graph of Gibbs free energy available to a bacterium at a pH below 7 created by Lema et al.[206]

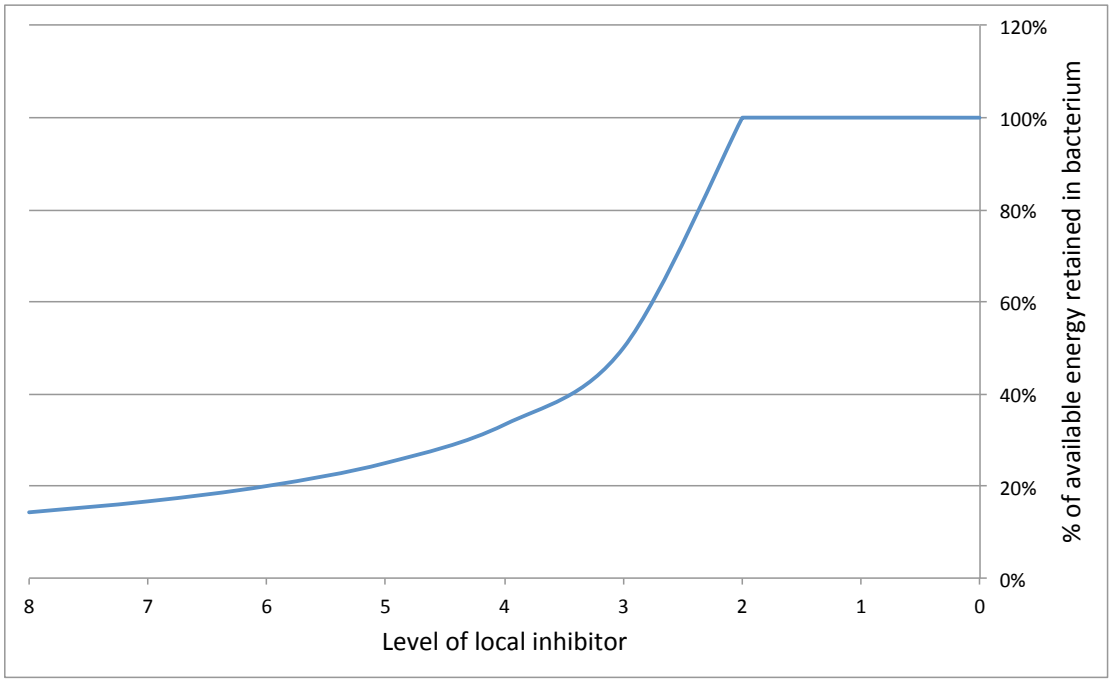


Figure 44 - Illustration of the proportion of total energy released by digestion of cellulose that is retained by a bacterium as a function of the local level of inhibitor - itself a proxy for local pH.

Model Operation

The model was operated as a series of batch runs, each comprising a single experiment varying one key parameter with associated subsidiary changes as needed. Given the number of potential variables, and the large number of experiments run, data from only a selection of typical experiments are set out below.

10.3 Experiments

Setting default parameters

The first experiment was to establish 'reasonable' parameters for the model, in order to have a default set that could be used as a basis for variation in further experiments. All subsequent experiments use the default set with declared modifications. The key variables and their default values are set out in Table 14.

Table 14 - Key input variables in the MATLAB hydrolysis model and their default values for the experiments described.

MODEL VARIABLE	Description	Default Value
T	Number of time steps before the model forces a stop. Only happens if the model fails to reach the target set in MINSUBSTP	500
L	Length of particle measured in matrix cells	50
W	Width of particle measured in matrix cells	50
D	Depth of particle measured in matrix cells	32
LSR	Liquid to solid ratio at start.	5
LP	Lignin %	10%
LL	Average length of a lignin chain (matrix cells)	5
NUMBACT	Initial number of bacteria	1 000
AR	Ratio of Activity of bacterium after compared to before the production of a daughter.	95%
DD	Number of daughters produced before cell death	10
CA	Number of activity units to digest 1 matrix cell of cellulose. A bacterium gets 1 activity unit per time step, reduced by the cumulative number of daughters and the level of local inhibition.	5
CD	Number of energy units the bacterium needs to have in store to produce a daughter. Each daughter reduces the stored energy units by this amount. Each unit of CA digested produces 1 unit of stored energy.	1
INHFUN	The function, chosen from a user defined list, to be used in any run to map the level of inhibitor at a point to the level of inhibition of bacterial activity at that point.	1
INSENS	A multiplier for INHFUN to increase or decrease the sensitivity of bacteria to inhibition.	0.4
DIFFN	The number of diffusion steps the model runs for each time step. The model carries out a diffusion calculation across the entire model, then repeats it DIFFN times before the next time step. It is an approximation to the solution of the equations needed to model diffusion accurately.	5

MODEL VARIABLE	Description	Default Value
DBL	Depth of the boundary layer from the surface of the particle	3
VBG	The background concentration of inhibitor above the boundary layer. Layers above DBL have the inhibitor concentration of VBG.	1
PS	Probability of a bacterium that arrives at the solid liquid interface actually settling.	10%
PG	Probability that a bacterium will be grazed at random by protozoa in any time step	0%
PD	Probability that a bacterium that has no cellulose left in its position, AND has no substance (lignin or cellulose) above it can detach and return to the menstruum.	50%
PRD	During rumination all bacteria can be detached. This sets the probability that any one bacterium will detach during rumination	30%
RUMINATEF	Number of time steps between rumination events	0
RUMINATEN	Maximum number of rumination events allowed in a run. -1 implies 'No Rumination'	-1
MINSUBSTP	The amount of cellulose left in the model at the end of the run (unless ended previously by T).	15%

The default parameters for both versions of the model (i.e. V20 and V20a) are identical. Figure 45 shows the cumulative digestion graph for a particle using all the parameters in the default parameters file. This simple curve reflects a complex set of activities and inhibition within the particle. A direct indication of the heterogeneity can be seen from the graphic in Figure 46 showing in cross section the levels of inhibitor in the particle at T=100.

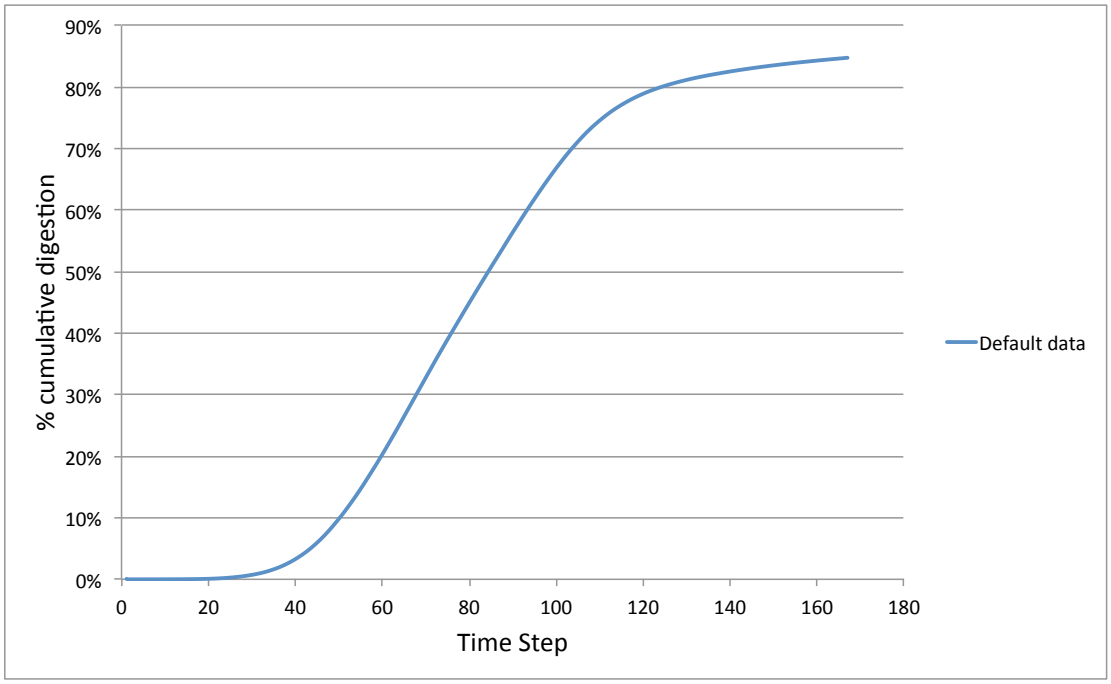


Figure 45 - Graph of cumulative digestion over time for default parameters used in both versions of the Matlab model.

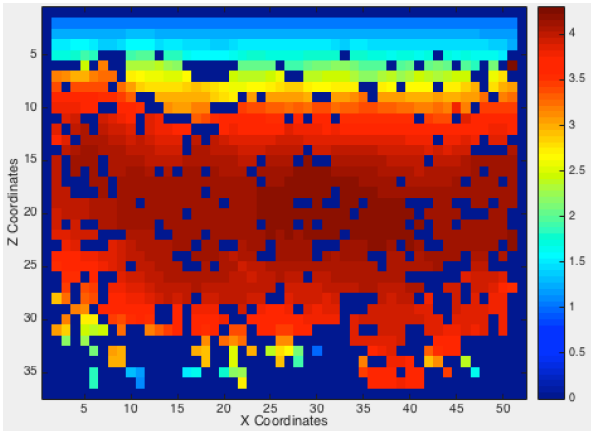


Figure 46 - Heterogeneity of inhibitor distribution within the particle at T=100. Dark blue cells with no inhibitor are either undigested or lignin; dark red have the highest level of inhibitor. The top three layers are the liquid boundary layer, showing reducing concentrations as the distance from the particle increases.

Figure 47 shows how the biofilm grows over time, forming distinct areas with different growth rates, reflecting the heterogeneity of the environment within the film.

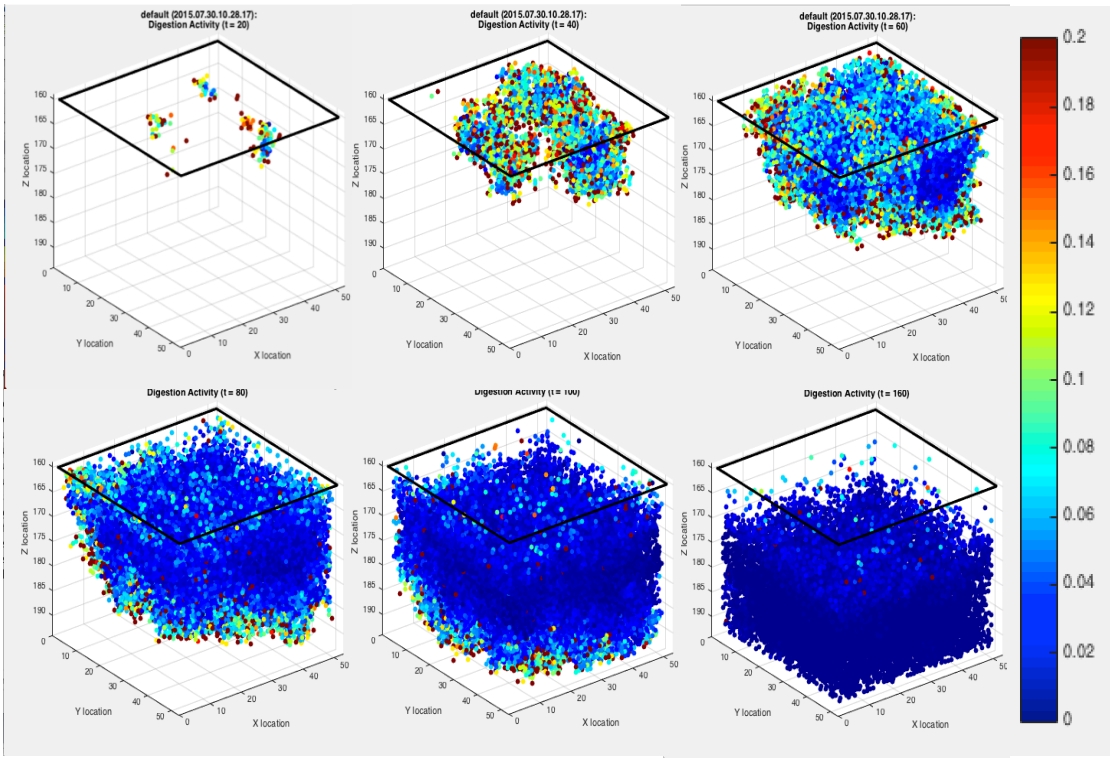


Figure 47 - Development of the biofilm for the default model settings. Graphs are for T= 20,40,60,80,100 and 160. Inactive cells are white, active cells are coloured as per the scale at the RHS. T80 and T100 show distinctly the existence of an active layer at the top of the biofilm where diffusion of inhibitors is least constrained, and at the bottom where colonisation by young bacteria of new spaces is taking place, and where there is limited inhibition. The centre of the film, dark blue, is relatively less active. Lighter colours tending towards red indicate increased activity.

THE EFFECT OF CHANGING THE INHIBITION CONSTANT

The graph in Figure 48 shows the effect of changing the inhibition constant from zero (i.e. no inhibition) to a high degree of inhibition. It shows behaviour that would more or less be expected, namely a progressive decay in the rate of digestion as the level of inhibition increases.

In order to explore the effects of a different sort of inhibition as proposed by Lema – i.e. where bacteria expend a large proportion of their energy to move VFAs out of the cell wall and therefore have less to use for the formation of new biomass, the analysis was repeated using model V20a.

Figure 49 illustrates the results. It shows that at the higher levels of inhibition the additional function makes little difference to the rate of digestion. However as the level of inhibition is decreased an altogether different form of the graph takes over. At the lowest levels of inhibition the overall rate of digestion becomes dominated by the inability of the bacteria to retain energy for growth.

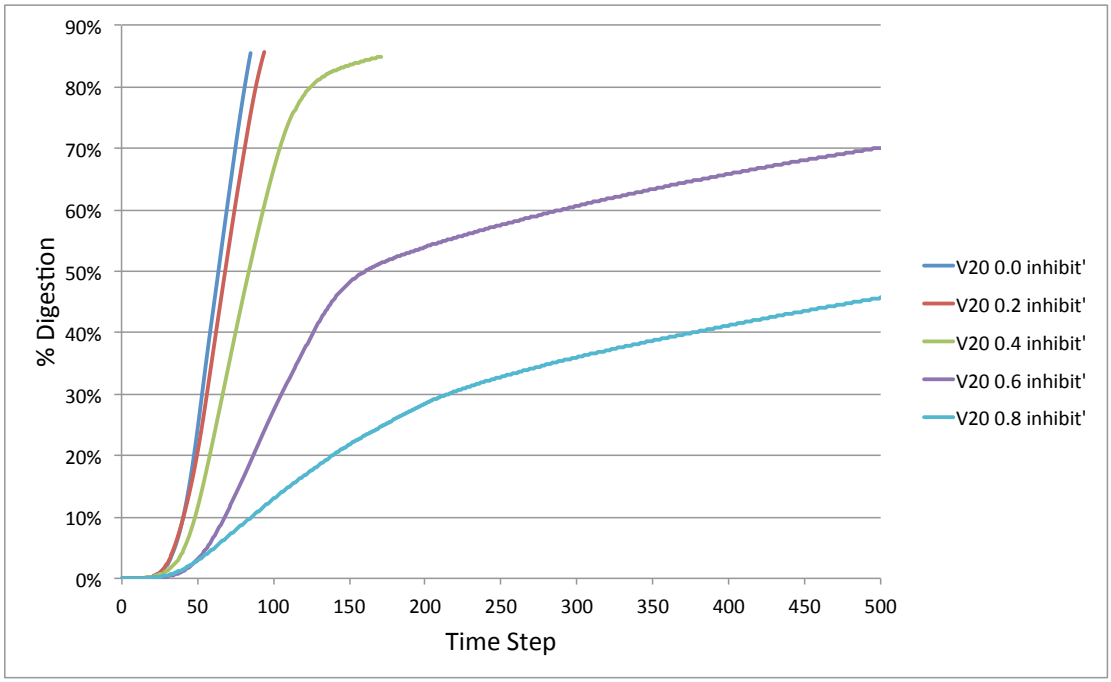


Figure 48 - Cumulative digestion over time, as a function of the degree of inhibition applied

More surprisingly, although lower levels of inhibition result in higher initial rates of digestion, overall rates of digestion become slower.

The pattern is striking, and consistent, and somewhat difficult to explain, though at its core is the fact that, as modelled, a bacterial cell which is uninhibited will digest cellulose rapidly to produce inhibitors (VFAs) faster than they can diffuse away. This will reduce the proportion of energy available to grow new daughters, and thus seems to stifle biofilm growth.

Clearly the model itself contains so many unfixed parameters, and so little detail of the specific mechanisms that are involved in real biofilms can be predicted from this result - save the one observation as follows. Inhibition of cellular activity, and inhibition of cellular growth (but not digestion) are both plausible mechanisms for constraining the growth of biofilms, and they may both have very different effects on the performance of the biofilm.

THE EFFECT OF INITIAL BACTERIAL CONCENTRATION

Bacterial settlement and colonisation of the substrate has been identified as a possible important factor in the effectiveness of AD. This suggests that the concentration of free bacteria in the liquor in a digester should have an impact on the rate of digestion of any feedstock.

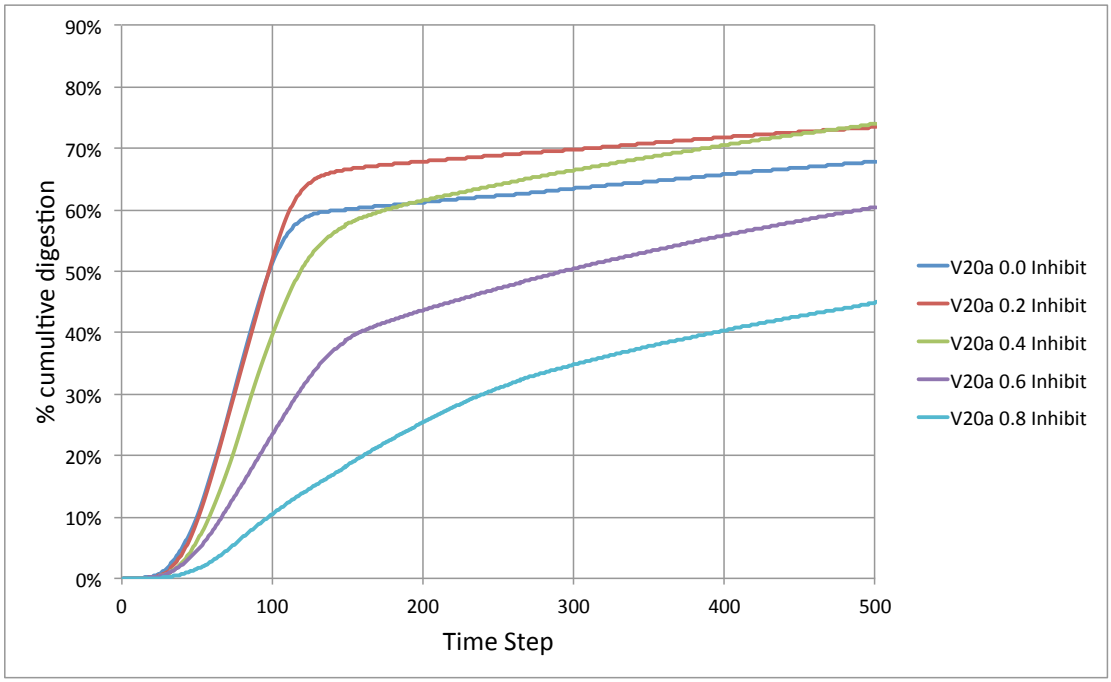


Figure 49 - Cumulative digestion over time with energy retention by bacteria reduced as a function of local inhibitor levels. This mimics the effects described by Lema with low pH and lactate. At lower levels of INHIBIT (which inhibits overall bacterial activity) the need to use energy simply to continue functioning rather than to grow new biomass becomes dominant.

This experiment models a wide range of initial free bacterial concentrations, ranging from 10 to 10,000 bacteria with all other conditions the same. Figure 50 shows that, as might be expected, the time to digest 85% of the substrate increases as the number of starting bacteria decreases.

Re-examination of the data, though, illustrates a rather different and less intuitive point. Figure 51 shows the same data, but aligned to the time that each experiment completes digestion of 85% of the cellulose available. Somewhat surprisingly, this shows that the actual digestion, once colonisation is established, is almost completely independent of the concentration of bacteria in the menstruum.

The reason for this phenomenon can be found in the ratio of settlement sites on the surface of a particle compared to the number of sites within the particle. Similarly, in a particle of plant material, once colonies are established, they are able to grow rapidly into the particle, harvesting fresh nutrients and occupying new sites. Establishment, however, is highly dependent on initial bacterial concentrations. It suggests that optimisation of the rate of hydrolysis requires the quite separate optimisation of a settlement phase, and a digestion phase.

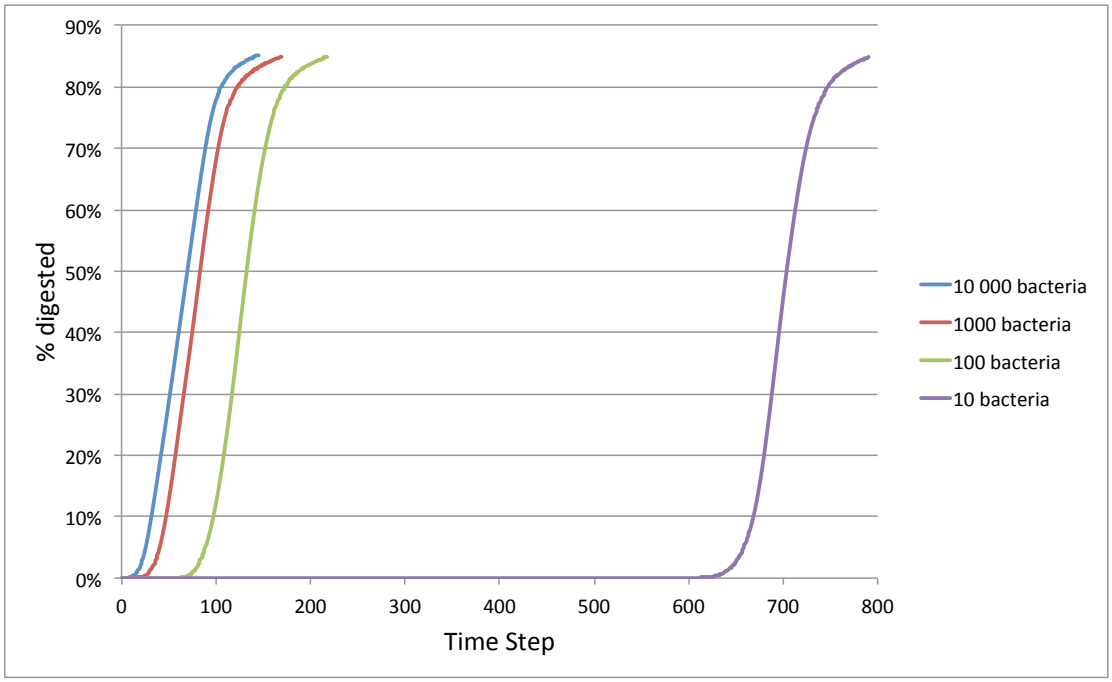


Figure 50 - Graph of the cumulative digestion over time of a sample with widely varying initial concentrations of bacteria.

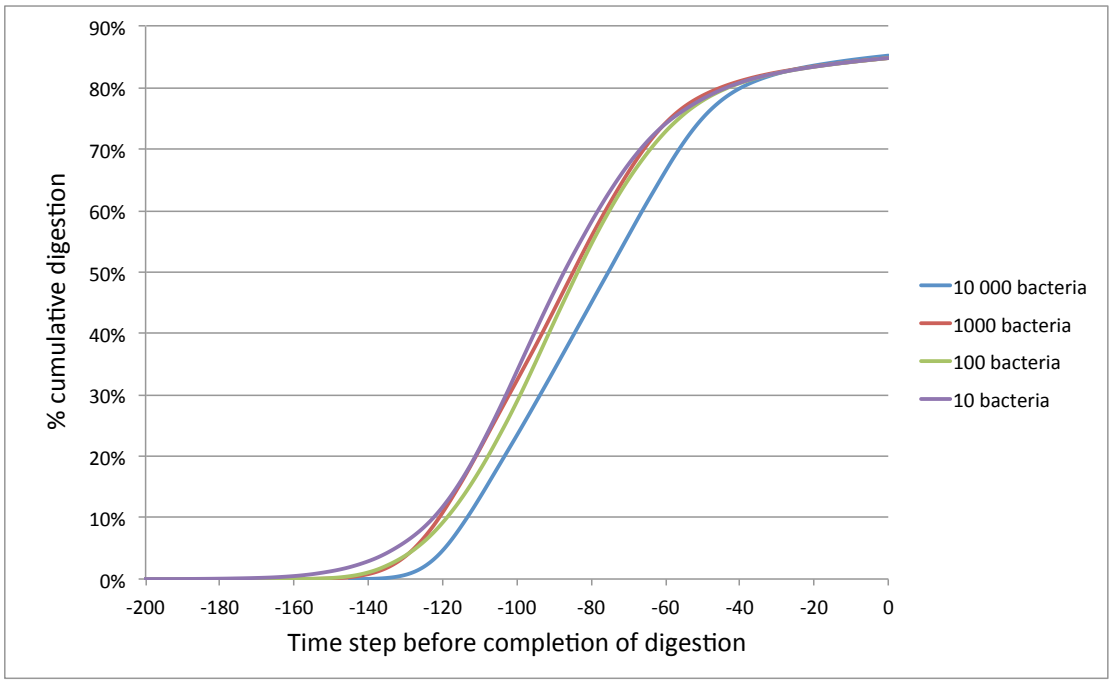


Figure 51 - Graph of the cumulative digestion over time of a sample with widely varying initial concentrations of bacteria - but with the time steps aligned to the 85% digestion point.

THE IMPORTANCE OF SETTLEMENT TIME AND VOLUME REDUCTION

In a traditional CSTR, the bulk of the volume is liquid, and this remains largely unchanged throughout the period of reaction. In a rumen, in contrast, the bulk of the material is solids, with only limited amounts of interstitial liquid present. If the volume of each particle decreases as

digestion proceeds, then it makes room for new feedstock, further increasing the volumetric efficiency of the rumen.

Figure 52 shows the digestion rates seen in the default case described above. The blue line shows the rate of digestion as a function of the original volume of the particle. This has two apparently unproductive periods – an initial one when settlement is taking place, and a final one when the digestion rate has decayed significantly. This is similar to the case of a particle in a CSTR where the volume is dominated by the more-or less unchanging liquid volume. The red line, on the other hand, shows the same data assuming the volume occupied by the particle decreases in line with its mass. This has the same unproductive period at the beginning, but it has both a much higher digestion rate per unit volume and even in the final period when the absolute digestion rate has decayed, the digestion per unit volume stays relatively high because of the loss of volume.

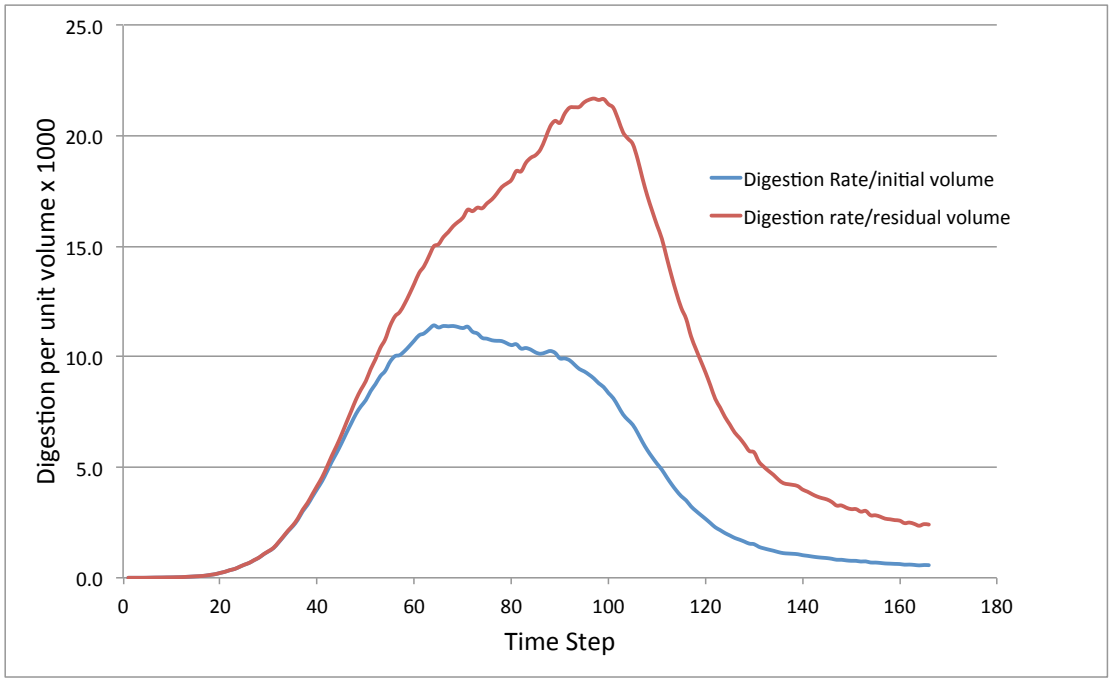


Figure 52 - Graph of digestion rates for the default case, showing the difference between raw digestion rate as a proportion of the initial volume of the particle, and the digestion rate as a proportion of the residual volume of the particle. The former is closer to the situation in a CSTR, the latter is more similar to the rumen.

Of course, in the real world, the volume of the particle may not decrease with mass as the lignin continues to give it structure, as does the indigested cellulose. This may point to a further function of rumination, and also of the muscular contractions of the rumen. Both of these may

serve to compress partly digested particles – making the rumen more efficient than it would be if the particles were undisturbed.

This also indicates the huge importance of the settlement period. During the time a particle spends developing a biofilm it occupies a maximum volume in the rumen, making it the most expensive phase of the process.

THE EFFECTS OF RUMINATION

Rumination is clearly important to ruminants. One of the effects of rumination is the increase in surface area of the particles of feedstock, and this is often described as its purpose. Sharma's data, as represented in Figure 34, however shows that simply increasing surface area has limited effects on the rate of hydrolysis. This experiment examined rumination using three separate cases with version V20a of the model:

1. A single particle as the reference case
2. The same particle, but cleaved horizontally so that it had twice the surface area. The liquid to solid ratio was also halved to reflect the fact that the average distance of a bacterium in the menstruum from a particle surface would be halved if the surface area was doubled.
3. The same particle, but with a rumination step at T=120. Rumination in the model has the effect of doubling surface area and halving the liquid to solid ratio, exactly as is done with the 'double surface area' experiment above. It also results in detachment of a large proportion of the bacteria, and removes all the accumulated inhibitor in the particles. This is consistent with a ruminant which chews particles to increase surface area, bathers them in alkaline nutrient broth, and shears quantities of bacteria off the particles to free them for re-colonisation.

The graph in Figure 53 shows how rumination, by doubling the surface area part way through digestion of a particle, significantly outperforms doubling of surface area at the outset. The reason is that by the time rumination happens bacteria are well distributed throughout the particle, but held back by inhibition. Doubling surface area at that stage, as well as detaching

some bacteria to create new colonies and invigorating existing colonies by neutralising inhibitors is, within the constraints of this model at least, very effective at increasing digestion rates.

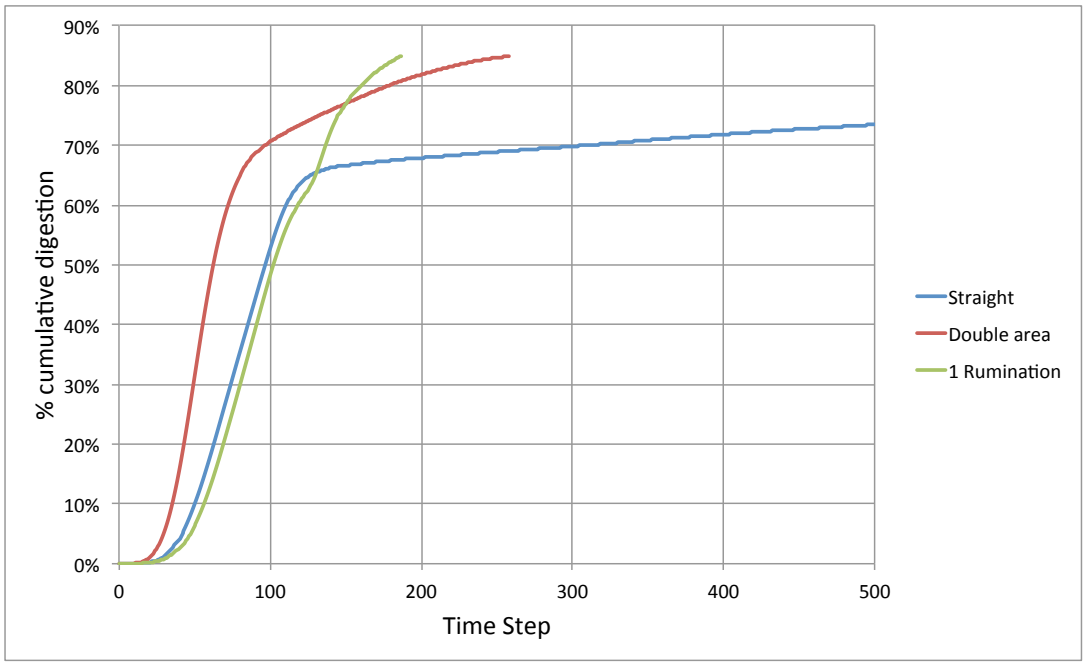


Figure 53 - Demonstration of the effects of rumination to double surface area during digestion as compared to doubling surface area at the start of digestion. The blue line shows the cumulative digestion of a particle over time. The red line shows the cumulative digestion over time of a particle half as thick and with twice as much surface area, and with half the liquid to solid ratio to reflect the increase in surface area with no change in liquid content. The green line shows the same particle with a rumination step at T=120. Rumination doubles surface area and halves the liquid to solid ratio as above, but it also resets the level of inhibitors to zero, and detaches a large proportion of the existing bacteria to enable new settlement to occur.

The limitations of the modelling mean that it is not easy to replicate the increase in boundary layer thickness part way through a run at the time of rumination. As a result the boundary layer thickness has been maintained the same for all runs at all stages. Had it been possible to double the boundary layer thickness to correspond with the smaller particle sizes a further effect would likely have been seen – with the option of doubling surface area at the beginning of the process performing even worse compared to rumination.

THE EFFECT OF BACTERIAL AGING

Bacteria age, and the aging of mother cells results in slower reproduction. This has been observed directly by Lawrence.[212] In one example, the doubling time of bacteria from a single

mother increased from 0.69 hours in the first generation to 5.2 hours in the fourth generation, equivalent to a 60% decline in activity per generation of daughter.

To investigate this, two sets of three models were run. Each set comprised three different factors for the loss of vigour after each generation of daughters; no loss, 30% loss per generation, and 60% loss per generation. The first set assumed no rumination, and the second set assumes one rumination step at T=120. The results are presented in Figure 54.

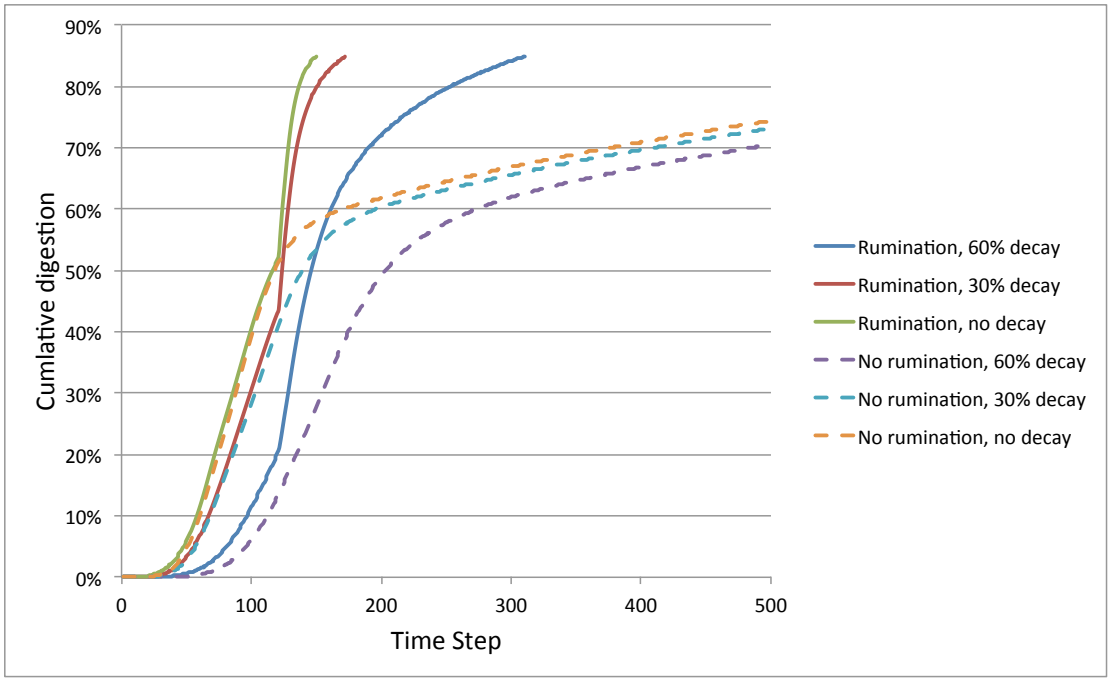


Figure 54 – Plots of cumulative digestion over time, with differing levels of decay of bacterial vigour between generations. 60% decay reflects the level of loss of vigour seen in each generation as reported by Lawrence. Two sets of data were modelled, one with rumination at T=120 (solid lines) and one with no rumination (dotted lines).

In all cases loss of bacterial vigour between generations has a material impact on the rate of digestion, though the difference between 30% decay and 60% decay is more significant than between 0% decay and 30% decay. The most striking difference, however, occurs when rumination is present. This suggests that for a ruminant, ensuring that the average age, and thus vigour, of the population of bacteria is important to maximise the benefits of rumination.

10.4 Modelling Summary

It is possible to play endless tunes with the model, and show all manner of subtle effects. However the reliability of the results is highly questionable without considerable further

validation and parameterisation, and probably an order of magnitude more complexity. What the modelling does show are some broad themes as follows:

1. Settlement of bacteria on a particle, and biofilm growth within the particle, are independently crucial processes that probably need to be optimised independently.
2. In a reactor where most of the volume is occupied by particles rather than liquid, the speed of establishment of the biofilm is critical to maximising volumetric efficiency.
3. The bacterial population in a particle may increase faster by growth of established bacteria rather than by settlement of new ones. This would favour rumination, which creates new particles or surfaces with already established populations, over excessive chewing at the time of eating, which create the new surfaces or particles but without any established bacterial populations.
4. Inhibitory conditions within biofilms are likely to be the source of the ultimate brakes on the system. Different forms of inhibition can have very different characteristic effects. Rumination may be an important way of resetting levels of inhibition and thus re-invigorating biofilm development.
5. Maintaining a young and vigorous population of bacteria is important.

Chapter 11 DISCUSSION

Part 1 of this dissertation argues the case that a 60-70% reduction is needed in the capital cost of AD installations to make it a globally significant and competitive technology. Key to reducing cost is the reduction in the physical size of AD plant. However, whilst a search on Google Scholar for 'Anaerobic Digestion' generates about 480,000 paper references, of which about 20,000 date from the last four years, there have been only modest changes in the costs and capabilities of commercial AD plants over recent years. Some opportunities that have been explored for improvement are pre-treatment, chemical addition during treatment, modification of (biological) plants such that they are easier to digest, and new bacterial communities. The likely large scale success of these is constrained for a range of reasons, including cost and complexity.

Given that current commercial technology for the digestion of lignocellulosic material only achieves a fraction of the hydrolytic speed of a ruminant, the apparent lack of progress is surprising. If even a fraction of the speed of hydrolysis of a ruminant could be achieved the cost of AD plant could be substantially reduced. Clearly there are factors operating in a ruminant or kangaroo which are not well emulated in commercial AD plants, and which need to be identified.

The remainder of this chapter draws together the elements of the discussion so far, to try and explain in part, at least, why a CSTR is so bad at hydrolysis and why a cow is so good at it. It draws some supporting evidence from the behaviour of macropod marsupials. The marsupial lineage separated from the placental lineage perhaps 40 million years before grass evolved, and never shared a herbivorous common ancestor. Thus areas where the lineages have convergently evolved similar digestive chemistry and behaviour are powerful indicators of factors that could be critically important to high rate digestion.

11.1 COMPARISON OF RUMINANTS AND AD REACTORS

WATER AND PARTICLE VOLUMES

The most obvious difference between the ruminant and the AD plant is the relative absence of water in the rumen. In a traditional AD plant a mix of particles and water is maintained and

stirred until the two components leave together. Because the VS represent only a small part of the total volume of this mix, the volume of water contained in the solids that is added to the plant during feeding remains more or less constantly present during digestion. In the rumen, on the other hand, the fresh feedstock forms a dense mat of large particles, and as it digests and liquid is released the excess liquid is flushed out, so not only is there less volume because there is less water overall, but also the volume occupied by a quantum of feed declines as the feed is digested.

Although the mass of a particle reduces over time, if it is not compressed by chewing or some other means, the structure of the lignin and undigested cellulose may ensure that its apparent volume is greater than the solid volume, effectively making it a water filled ball of fluff. This will reduce any density difference between particle and liquid, and also increase the diffusion path for nutrients into and inhibitors out of the particle. This describes the situation in an AD reactor. However in a ruminant, recycling the material through the mouth and compressing it will reduce its volume, and increase its density. In a kangaroo, which doesn't ruminate, peristalsis in the long thin fore-stomach possibly has some of the same effects.

Thus the simple mechanical arrangement of the ruminant stomach and its water content certainly account for a proportion of the volume saving in a ruminant.

STIRRING, MIXING AND FLUSHING

In an AD plant, for the reasons discussed previously, stirring becomes less effective at clearing inhibitors from biofilms, and supplying nutrients, as the particles become smaller. This is a possible explanation for the apparent underperformance of the hydrolysis of smaller particles as seen in the re-interpreted Sharma data. The problem is compounded by the tendency in AD plants to create liquid that contains large numbers of small particles – raising its viscosity.

The ruminant takes a different approach. Feedstock in the rumen consists of a mix of particle sizes forming a thick mat, permeated around once a minute with a liquid of viscosity that appears similar to water, driven by ruminal contractions[157,160]. The thick mat effectively holds the particles almost stationary relative to the liquid which is moved by peristalsis. This means that the liquid flows along complex narrow paths, with maximum contact between liquid and solid,

and plenty of turbulence to ensure advection. The mat of coarser particles will also serve to trap many finer particles within the structure – so ensuring that they are also well washed.

Knutsen et al. demonstrated the importance of retention of enzymes in a reactor by filtration of the flushing liquid and return of the retentate.[136] It is not clear how much free enzyme is present in in the menstruum of a rumen, but it is clear that there is a high concentration of planktonic bacteria.[176] Given the observation that rumen bacteria lyse easily, retaining them in the reactor or rumen is crucial.[125] In ruminant digestion mixing by contraction is decoupled from the flushing that occurs as liquid passes through the rumen. Mixing is frequent, and transfers VFAs to the rumen lining where they are removed by absorption. However planktonic bacteria and free enzymes are retained in the rumen and not flushed out by this process.

Cattle other than for milk might drink 25-50 litres of water a day, and produce 100-200 litres a day of saliva.[213,214] All of this material must pass through the rumen and flow out – giving an average flow rate of around 8 litres/hour. Assuming a rumen volume of around 180 litres for the reticulorumen and omasum combined, this gives a flushing rate of .044 hr^{-1}, or around 1.1 day^{-1}. This is many times lower than the flow rate created by ruminal contractions at 1 minute intervals (1,440 contractions per day of the entire liquid volume). However it is also many times greater than the flushing rates in an anaerobic digester, which could be around 0.1 day^{-1} or less.

O'Sullivan noted that, in a series of comparative batch studies on anaerobic digestion, that rumen inoculum contained significantly more microbial cells than inoculum from more conventional AD sources[176].

Thus, ruminants achieve much higher levels of mixing and flushing than AD reactors, yet retain much higher concentrations of bacteria. Some of this is achieved by decoupling mixing, VFA removal and flushing. However there must also be some factor in ruminants that stimulates the growth of planktonic microbes, as if the different sources of inoculum had identical rates of growth the rumen could not sustain equilibrium, let alone show a higher population.

MANAGEMENT OF BACTERIAL AGING

The analysis presented in Chapter 10 shows how bacterial aging may change the vigour anaerobic digestion significantly – a likely situation in a CSTR. It has already been noted that ruminants must be able to encourage bacteria to reproduce rapidly. As well as providing the population to enable rapid settlement, this rapid growth will also ensure that the average age of the population is kept young, so any loss of vigour resulting from aging is minimised.

SALIVA AND RUMINATION

AD plants generally operate using no more than the chemistry provided by the process itself. Cows, on the other hand, add bicarbonate, phosphate and small amounts of other components including ascorbic acid to their rumens via salivary excretions. The similarity between kangaroo and ruminant saliva, and its difference from the saliva of non-grazers suggests that this is important to cellulose digestion.

There are several possible explanations for the quantity and quality of saliva in ruminants:

1. The high rate of hydrolysis in a rumen derived reactor could overwhelm any autogenic bicarbonate production or buffering. Gijzen et al. demonstrated that buffering with $NaHCO_3$ fed continuously at 2.2g/l at a rate of 2 fermenter volumes per day enabled hydrolysis rates using a rumen inoculum of up to 25.8gms VS/l/day. At that rate, using their two stage process, failure to break down propionate became the limiting factor.
2. Inorganic phosphate is an important buffering agent, but also an important agent in the formation of biofilms.[215] Its presence in the saliva, where bacteria are being sheared off existing particles, and new surfaces are being created, may be highly significant.
3. The high alkalinity of saliva coupled with mechanical action may effectively neutralise VFAs in the biofilms on particles being ruminated. Lema's work on lactate suggests that pH within a biofilm is critically important. It may be the case that a low pH actually works in favour of hydrolysis, by forcing bacteria to use many glucose molecules, and thus produce many acetate molecules, in order to support normal metabolic activity. This would move the balance away from producing new cellular biomass, and towards producing maximum acetate. However in the mouth, a high phosphate level and high pH

coupled with new surface area, may well be ideal for major expansion of the microbial population. These new microbes would either settle on the new surface area created by rumination, or alternatively be flushed back into the rumen. Thus the heterogeneity of the mouth/rumen system may well be an important factor in ruminant hydrolysis.

4. Ascorbic acid may well be a crucial, and overlooked component of ruminant saliva. There is clearly evidence for its potential as an accelerant of anaerobic hydrolysis, yet there seems to be no reference in the AD literature to its effectiveness or otherwise.

DECOUPLING OF ACIDOGENESIS AND METHANOGENESIS

AD reactors traditionally encourage acidogenesis and methanogenesis in the same vessel. This requires the cellulolytic and methanogenic phases of the reaction to be in balance. However the work of both Mumme and Gijzen showed that high rate reactors work better if methanogenesis is in part at least separated from acidogenesis.[116,178] Gijzen, working with rumen fluid as inoculum, found that at digestion rates of over 25g VS/l/day failure of propionate degradation was the reason for a decrease in methanogenesis.

In ruminants, acetoclastic methanogenesis is largely suppressed by absorbing all VFAs through the rumen and omasum walls, removing a major potential restriction on the rate of cellulolysis.

11.2 CONCLUSIONS

This section started with the conventional assumption that cellulolysis was the rate limiting step in anaerobic digestion. The work presented here now suggests that, whilst this may be true for conventional digestion, it is probably not true for a high speed system that approached the speed of hydrolysis of a ruminant. In both the Mumme and Gijzen reactors, either acetogenesis from propionate or acetoclastic methanogenesis would seem to be limiting. This problem can be overcome by separation of the reaction phases, but the short life of cellulolytes and the requirement for high flushing rates leads to the possibility that some form of membrane separation is required to contain cellulolytic microbes within the hydrolysis phase whilst removing the VFAs for downstream processing. An alternative possibility is that high flushing rates and rapid removal of cellulolytes could lead to strong selection pressure in favour of fast growing and fast settling 'pioneer' genotypes – thus promoting a high hydrolysis rate.

A major obstacle in untangling the factors that differentiate AD from ruminant digestion is that the focus of most AD research to date has been on manipulating the bulk environment of the reactors. However it is the micro-environment experienced by the microbes that determines the rate of reaction, and Chapters 9 and 10 have demonstrated that this micro-environment is only loosely related to the macro-environment.

Understanding the micro-environment and how a cow manipulates it is likely to be one key to high rate AD. In this context, the observation that changing the micro-environment surrounding biofilms by changing boundary layer pH may favour either microbial growth or acidogenesis merits considerable further investigation. It may offer part of the explanation of how a cow sustains a high population of young and vigorous cellulolytes and ensures that they settle rapidly on the feed particles in spite of the rapid transit of liquids and solids through the rumen. It is possible that rumination plays an important role by providing a different mechanical and chemical environment in the mouth to that in the rumen, with the mouth providing an environment conducive to reproduction and settlement, and the rumen providing a rather different environment favouring acidogenesis.

No discussion of ruminant digestive efficacy can be complete without considering the role of saliva. The degree of convergent evolution between the saliva composition of macropods and ruminants provides strong evidence that this is a crucial factor in the success of high speed hydrolysis. The bulk inorganic chemistry of saliva has been examined in the literature, but the more cryptic organic components have not. In particular, ascorbic acid is a known promoter of anaerobic cellulolysis but its presence or significance in saliva other than in ruminants appears not to have been investigated, nor has its possible role in AD.

In conclusion, there is almost certainly no 'silver bullet' that explains the failure of commercial or laboratory AD systems to match animal models, and thus could be used to deliver high speed Advanced Anaerobic Digestion. The reality is probably that there are multiple differences in the two systems, each of which contributes a modest proportion to the efficacy of the animals, but when multiplied together create a substantial net improvement in performance. This multiplicity of differences between AD and animal models of hydrolysis may make it difficult to replicate the full efficacy of the ruminant, but it provides numerous avenues for research and development to

make modest improvements. A significant number of these differences are identified in this research, and only a proportion of these need to be effective to achieve or exceed the power target initially established – namely 1kW(e)/m^3.

Chapter 12 FUTURE WORK

This research so far has described a range of important factors that, if validated experimentally, could significantly increase the power density and economic viability of commercial Anaerobic Digestion. Delivering these improvements will require a suite of new techniques to be applied to AD research, ranging from new reactor designs to investigative techniques such as confocal Raman spectroscopy. It will require a multi-disciplinary approach involving chemistry, microbiology, membrane filtration, animal research, and systems control. Achieving this is beyond the scope of this research, but will form the focus of forthcoming work.

This chapter describes a vision of the future work needed. It includes the design and construction of a new laboratory reactor type together with its balance of plant, that will allow many of the functions of a ruminant digestive system to be investigated and evaluated. It is intended that this design will be used to begin an intensive research phase over the coming years. The design therefore is less of a fixed piece of engineering and more of a tool kit that can be easily and cheaply modified as experience is gained.

12.1 OBJECTIVE AND STRATEGY

The explicit purpose of future work is to get a sufficient pragmatic understanding of the digestive mechanism of ruminants and other grazers to allow a power density of in excess of 1kW(e)/m^3 of hydrolyser volume to be achieved in a commercial plant. In practical terms, this suggests perhaps a target of >2kW(e)/m^3 to allow for losses in translation from laboratory to commercial process. Based on the previous estimate of 1.2MWh/dry tonne, 2kW(e)/m^3 requires the hydrolysis of approximately 40 gms dry matter/litre/day. To recap, a cow achieves approximately 8.4kW(e)/m^3/day.

There are myriad factors that could explain some or all of the differences between AD and digestion. Although many of these have been described in previous chapters, the work is largely based on re-interpretation or re-purposing the experimental work of others, or on theoretical modelling. What is now lacking is a clear understanding and quantification of the impact of each of these factors and their interactions with each other.

Given the complexity of the challenge it is unlikely that a full understanding of the operation of ruminant or macropod digestive systems will be achieved in a useful time scale. What is proposed here, therefore, is a strategy with three parallel arms.

The first involves taking a simple pragmatic approach to establishing the impact of each identified factor in isolation and in combination with others, without necessarily understanding why it works. This needs a new reactor design that emulates the digestive function of a ruminant better than any previous AD reactor to trial different combinations of reagent, mechanical arrangements and control systems, and by a process of attrition and elimination, determine the contribution that each combination of makes to the goal of high speed hydrolysis.

The second arm of the study is to use the information gleaned from the first arm, and understand exactly what differences exist between the chemistry of the menstruum and the make-up fluid in the reactor, and that of the rumen and saliva *in vivo*. Whilst gross inorganic chemistry has been quantified (pH, bicarbonates, phosphates, some trace elements) in saliva, there has been little work done on potentially key organic components, (e.g. ascorbic acid), or to investigate what else might be present and significant. Furthermore the roles of these components have barely begun to be resolved. This is an area where comparative analysis of ruminants and placental grazers may offer important clues as to the relative importance of the different components. There is another possibly important animal to study, the Hoatzin. This is a South American rainforest bird known colloquially as the 'cow bird'. It is a leaf eating foregut fermenter that flies. To achieve this it must be exceptionally efficient at digesting plant matter.

The third arm involves taking a more fundamental approach to understanding the micro-environment in which digestion in biofilms actually operates. Chapter 10 demonstrated the complexity of biofilm growth with a single species and the extreme heterogeneity possible in these films. Multiple species occupying different niches, and consuming each others catabolites will make the position even more complex. Understanding these interactions is therefore a major objective of future research,

12.2 New Reactor Design

This section describes the design of a new reactor and its balance of plant, intended to replicate sufficient of the functionality of the digestive system of a grazer (ruminant or macropod) to allow individual factors that might contribute to digestive efficiency to be investigated and isolated, with the aim of developing a palette of strategies that could be incorporated in a commercial plant design.

Key features of a novel reactor

Hume (1989) describes ruminants fore-stomachs as CSTRs.[165] However this is somewhat misleading; there are very substantial differences between CSTRs and the way that a rumen works, and it is by exploring these differences that an approximation may be made of ruminant digestion. Some of these key differences may be summarised as follows:

- In a CSTR solids and liquids are thoroughly mixed, and thus leave at the same time. In a ruminant, solids remain in a relatively immobile mat until they degrade to small particles at which point they exit. Liquids both flow through the rumen independently of solids, and also are circulated through the solids bed by peristalsis. Both of these motions are separate from each other – given a much greater level of surface/liquid interaction than in a CSTR.

- Long retention times in CSTRs, and tendency to comminute feedstocks, means that the menstruum in a CSTR is relatively viscous. This viscosity aids with energy transfer from stirrers to the solid/liquid medium. In a rumen the high flow rate, relatively large particle size, and continuous removal of small particles means that the viscosity of the menstruum is low. This aids advection and the transfer of nutrients and catabolites between particles and menstruum.

- In an AD CSTR, or even a system with multiple series CSTRs, all reaction products remain with the menstruum until they separate out as gas. In the ruminant, the rumen walls are permanently removing liquid and VFAs, leaving solids behind.

- CSTRs promote homogeneity in the menstruum by stirring. In a ruminant there is a clear difference between the chemical and mechanical environment in the mouth and that in the rumen. Ruminants operate a highly heterogeneous system. Rumination selectively removes large particles from the rumen, manipulates the chemistry, the surface areas and the biofilms on them, and then returns them to the rumen. The omasum is different again.

The AD reactor with the greatest similarity to the rumen is that devised by Mumme et al. (2010), which comprised a solid bed retained in the reactor with menstruum flowing through it. The flowing menstruum was pumped out of the reactor to a series of methanogenesis reactors to remove any residual VFAs prior to recirculation.[116] The reactor designed therefore as the workhorse for the future work borrows from Mumme as a starting point, but adds a range of features and functionality relevant to ruminant digestion. Table 15 sets out in summary a comparison of the key characteristics of a traditional CSTR reactor, a rumen, and the proposed new experimental reactor

Table 15 - Comparison of key features of CSTR, rumen and proposed experimental reactor

CSTR	**Rumen**	**New Reactor**
High liquid to solid ratio so large volume reactor	Lowest possible liquid to solid ratio – effectively a packed bed	Lowest possible liquid to solid ratio – effectively a packed bed
Comminution usually only at beginning of process	Rumination at intervals results in shearing of biofilms and probable creation of a cloud of active bacteria for settling	Limited shearing during hydrolysis – limits set by nature of the reactor as a printed plastic unit
Buffering from internal chemistry during reactions	Large flows of phosphate rich, high pH saliva accompany initial chewing and rumination to stimulate cell activity and bacterial settling	Large flows of artificial saliva both during continuous operation and also to accompany feed material
Inoculum from other reactors with slow growing biomes	Inoculum usually from maternal biome	Inoculum from strained rumen fluid
Solids in suspension and	Solids locked in bed and liquid flushed	Solids locked in bed and

moving with liquid	over	liquid flushed over
Viscid liquid – low settling velocities so low turbulence	Inviscid liquid – higher settling velocities and higher turbulence	Inviscid liquid – higher settling velocities and higher turbulence
Mixing by stirrer – little relative movement of particles and fluid	Mixing by flow over fixed bed; significant relative movement of particles and fluid	Mixing by counter-current flow over fixed bed; significant relative movement
Long solids and liquids retention means no selection for fast growing organisms	Very short retention time of solids and liquids means strong selection pressure for rapid microbe growth	Solid and liquid retention time independently variable to test significance of selection pressure on digestion rates
VFA production rate limited by rate of VFA conversion to CH4	VFAs all absorbed by rumen lining to no VFA inhibition	VFAs continuously removed and pH controlled to ensure no acidification
Liquid fine particles and coarse solid retention times the same	Liquid , coarse solids and fine solids retention times all different	Liquid and solid retention times independent, fine solids largely go with coarse solids

REACTOR DESIGN

The reactor is built largely of 3-d printed componentry, using Selective Laser Sintering (SLS) of nylon) and acrylic tubing, with a minimal number of traditionally engineered components to allow low cost production.

Figure 55 shows a rendering of the assembled reactor. Annex B contains a series of detailed cross-sections. The reactor is essentially plug flow with material entering the bottom and exiting the inclined tube at the top. Liquid flows in through the top and exits the bottom through a fine printed sieve plate. A key feature of the reactor is the presence of a hexagonal drive shaft running from top to bottom, and half round stationary shafts set into pockets in the nylon base and upper portion. This allows the placement of a series of tools inside the reactor that can either be driven by the hexagonal shaft or alternatively fixed by locking onto the perimeter shafts. The vertical placement of the tools is secured by a series of spacers that can be placed at any point along the central shaft.

Material is fed in by being pushed down the feed tube and starts to rise as soon as gas starts to evolve. This is similar to the Mumme reactor. However as liquid flows from top to bottom in this reactor, rather than bottom to top as it does in the Mumme reactor, a rotating spinner at the bottom with angled blades helps the material to rise, and to ensure that it doesn't get drawn downwards by fluid flow. A range of spinners and stators can be added as needed to lift material, shear it, or otherwise mix it (Figure 56).

The top spinner, at the material exit, operates to push material downwards and inwards away from the exit under normal operation of the motor. However material can be removed by reversing the motor in which case the spinner is designed to lift material and move it radially outwards towards the exit tube. Solid material expelled from the top will be collected in a condom or similar elastic airtight container that fits over the exit tube lip.

Recirculated liquid enters the top of the reactor and fills a hydraulic lock – to seal the exit of the shaft from the reactor, and then flows out and over a plate covered with holes 500µ diameter so that the fluid spreads more or less evenly across the top surface of the reactor.

At the bottom of the reactor is a removable sieve plate perforated with numerous 200µ holes to allow liquid to flow out but to restrict any flow of particulate matter that might have sunk.

The liquid outflow line comes below the sieve plate. Above the sieve plate is a second fluid exit which is used to balance pressures within the reactor. This is described further under the 'Balance of plant' section.

All the internal tools are simple 3-d printed objects, and can be added, removed or re-designed at will. This provides flexibility to try and optimise the material flow, mixing, and shearing.

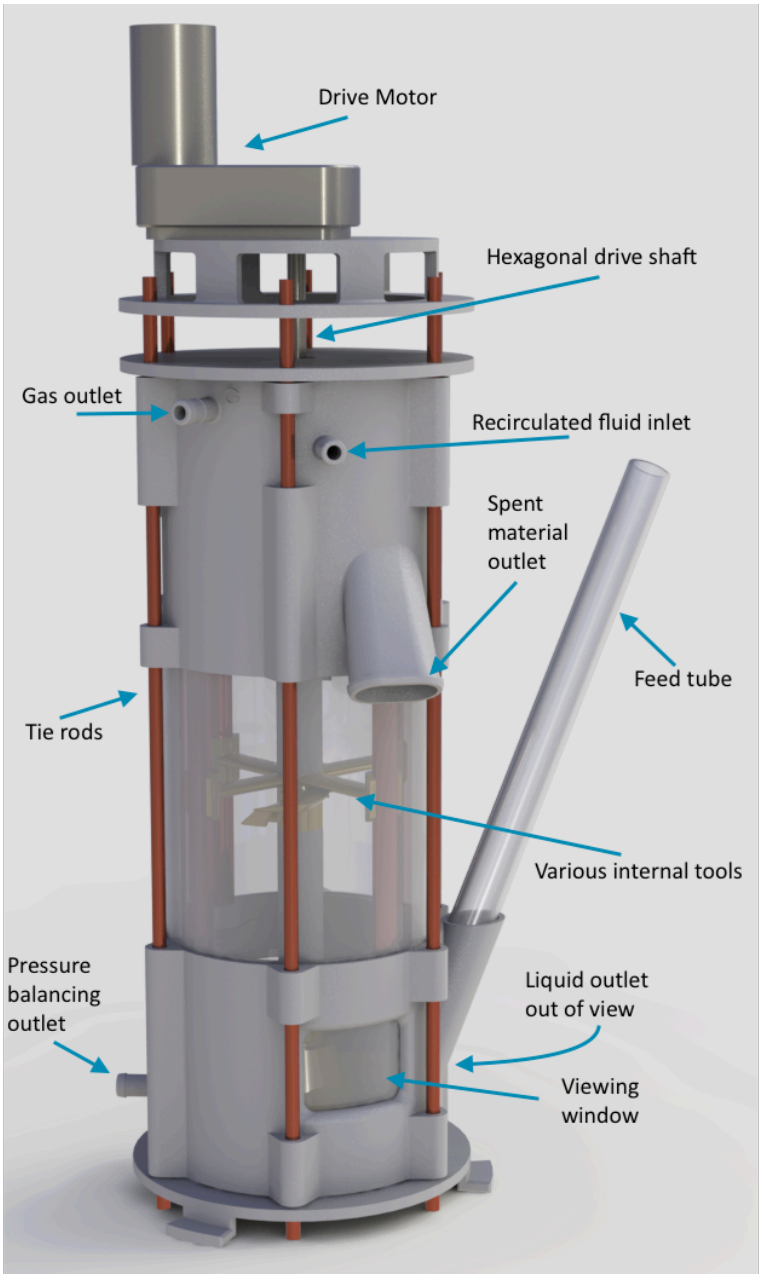


Figure 55 - External view of reactor. Matt grey components are in SLS nylon, transparent elements are acrylic tubing, brown are steel, and silver is motor for central drive shaft.

Cross-section drawings of the reactor, showing the central drive shaft and a selection of tools fitted are shown in Annex B. Tools can be slid up and down the central shaft and held in place by spacers. Torque for driven tools is provided by having a hexagonal boss that mates with the hexagonal drive shaft – stationary tools have circular bosses that rotate freely and are held in place by half round steel supports at the inner perimeter of the reactor.

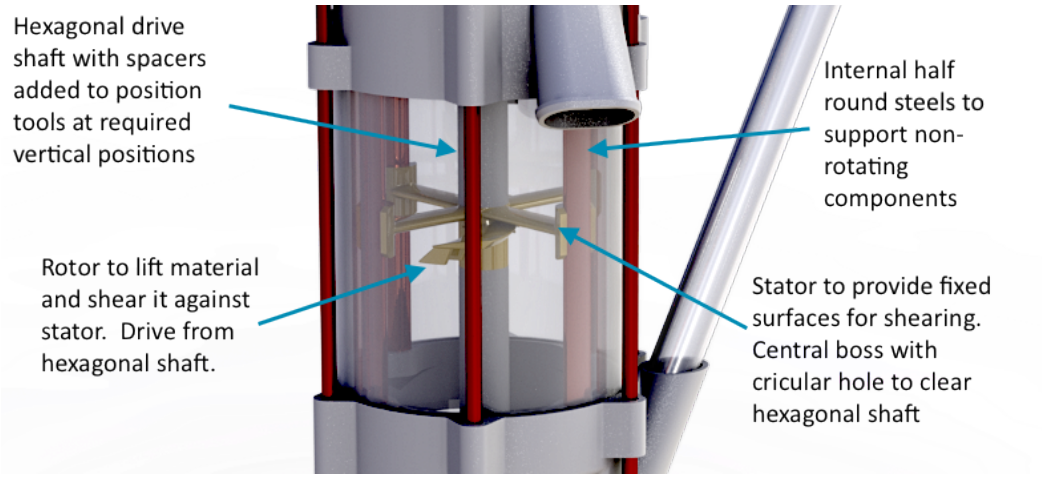


Figure 56 - Detail showing a stator fixed to the inside stationary vertical shafts, and a rotor driven off the central hexagonal shaft (both yellow) – to provide a degree of shearing to simulate the mechanical aspect of rumination. Other tools for lifting, shearing and tumbling materials can be added at will.

Balance of Plant

A ruminant has three forms of liquid flow: flow of liquid through the rumen originating from drinking water and saliva; flow of liquid out of the rumen via the membranes lining the rumen walls, and the periodic flow of rumen fluid around all the particles driven by peristalsis. To simulate this, liquid in the proposed reactor flows from top to bottom. It is drawn out of the bottom by a double-headed peristaltic pump, and past a side-stream membrane filter. Permeate is drawn through the filter by a second independent peristaltic pump, according to a control algorithm that can be varied as a result of experience, and into a flexible bag. The retentate is made up to volume by make-up fluid, and pumped back to the reactor top by the second head of the first peristaltic pump. By using a double head pump, the volume of liquid drawn out and returned are kept identical, with the balancing amount coming exactly from the make-up fluid.

The level in the reactor is critical to prevent material flowing out of the exit inadvertently. This is maintained by keeping the reactor in a water bath together with all permeate and make-up fluid kept in bags, and a third bag of DI water. This third bag is connected to a separate outlet at the bottom of the reactor, and allows small amounts of fluid to flow in and out to ensure that the level in the reactor is exactly the same as the level in the water bath. This ensures accurate and consistent control of levels throughout the system regardless of the addition or removal of feed

and the removal of permeate and the addition of make-up fluid. Key components of the balance of plant are shown in Figure 57.

12.3 Experimental work with new reactor design

Comparative digestion rates in a reactor

Operation of the test reactor requires an initial inoculum, and a reservoir of make-up fluid (the system's equivalent of ruminant saliva). Whilst experience and resources will constrain and modify the experiments actually performed, it is useful to set out an initial set of proposed experiments.

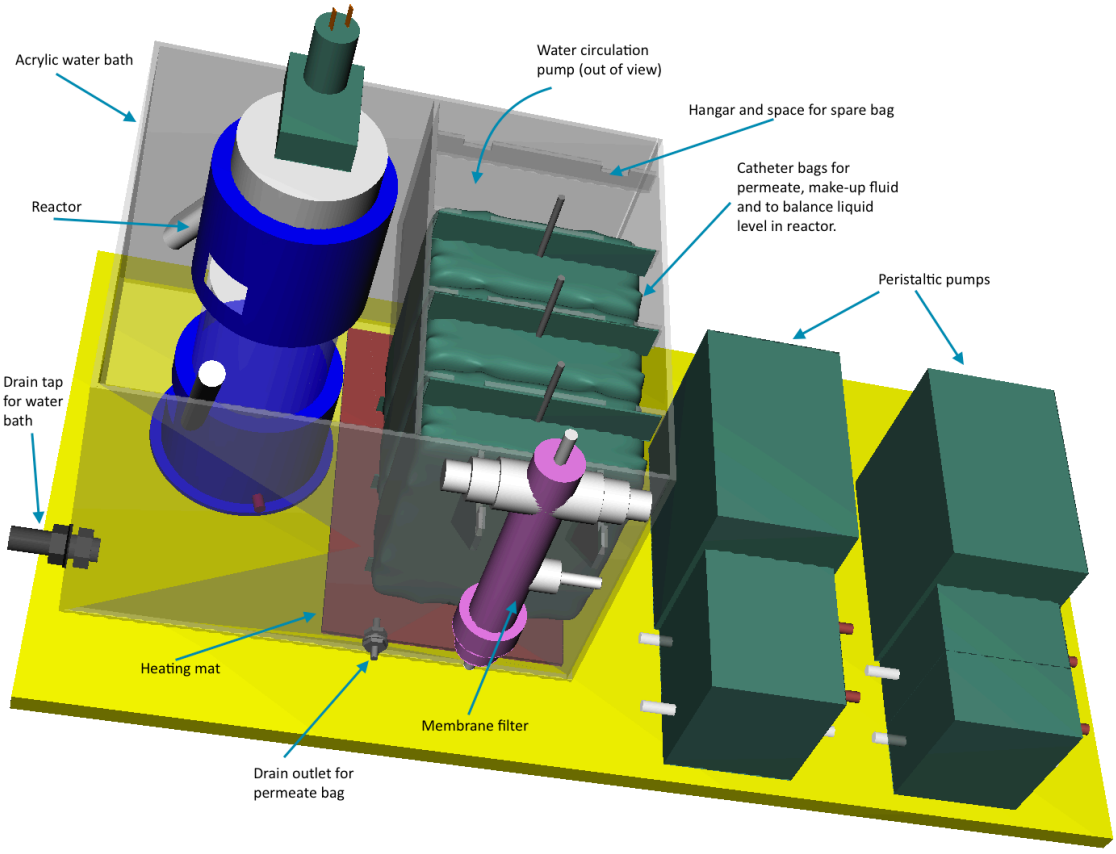


Figure 57 - Schematic arrangement of reactor and balance of plant, showing the principal elements. Lids have been removed from the water bath to better show the contents, and no interconnecting piping is shown.

The first set of proposed experiments is to compare the rates of AD in identical reactors with a range of different inocula and make up fluids. Table 16 sets out the initial proposed

combinations. Whilst many of these will be relatively straightforward to carry out, there may be challenges in securing saliva from suitably cannulated animals. This set of experiments, although lengthy, will allow the an assessment of the relative importance of each component of the system. The experimental work of Gijzen (1988) is closest to Experiment 5, and that of Mumme (2010) is closest to Experiment 9.[116,178]

Once data starts to emerge about the relative differences, and how those differences develop over time, detailed chemical and microbiological investigation to assess the differences between the pertinent inoculum or make up fluid compositions can be instigated.

Table 16 - List of first round of experiments to determine the relative importance of each component in an AD reactor.

Expt No.	**Feedstock**	**Inoculum**	**Make-up Fluid**	**Notes**
1	Rumen content	Rumen fluid	Rumen fluid	To provide a baseline and compare with digestion rates in vivo.
2	Rumen content	Rumen fluid	Ruminant saliva	To assess whether there are any differences between rumen fluid and saliva as make-up fluid.
3	Maize silage	Rumen fluid	Rumen fluid	To provide a baseline for maize silage, and to assess whether there is any difference between maize silage in vivo and in the reactor
4	Maize silage	Rumen fluid	Ruminant saliva	To assess whether the difference between expts. 1 and 2 is maintained using silage rather than rumen content.
5	Maize silage	Rumen fluid	Artificial saliva	To compare with 4 and determine whether there is a key difference between artificial and real saliva
6	Maize silage	Rumen fluid	DI water (+ key minerals)	To compare with 4 and 5 and determine how critical saliva of any form is to the rate of reaction.

Expt No.	Feedstock	Inoculum	Make-up Fluid	Notes
7	Maize silage	AD digestate	Ruminant saliva	AD digestate from a commercial digester.
8	Maize silage	AD digestate	Artificial saliva	
9	Maize silage	AD digestate	AD digestate	
10	Maize silage	AD digestate	DI water (+ key minerals)	

DIFFERING MECHANICAL AND FLOW REGIMES

Whilst both Gijzen and Mumme used simple reactors with no mechanical inputs to the digesting material, the evidence suggests that some degree of shearing and mixing is important. The new reactor design incorporates a range of powered internal tools for lifting, tumbling and shearing the material within the reactor at different points. These can be added, repositioned or removed as needed. In addition, there is a need to mimic the effects of the rumen lining be removing VFAs as they are produced. The rate of flow of the circulating medium and the rate at which liquid is drawn from the circulating medium and replaced by make-up fluid are separately variable in the proposed reactor.

12.4 BIOFILM AND MICROBIOLOGY INVESTIGATIONS

Ultimately, all cellulolysis occurs in the biofilms formed on the feedstock, yet there is little published literature on the nature and development of biofilms on lignocellulose. Part of the problem is undoubtedly the difficulty of probing films without disturbing them and thus distorting the features which the experiment is attempting to measure. New techniques, such as confocal Raman spectroscopy open up the opportunity to start to study these films to determine some of their key characteristics. The analysis and modelling described in Chapters 9 and 10 provide some initial targets for investigation.

The modelling in Chapter 10 illustrates the importance of rapid biofilm formation. The easiest, and thus probably the first target, is to understand what in the chemistry of saliva or the rumen best promotes the rate of settlement and formation of biofilms on lignocellulose. There is clear evidence in the literature for the role of phosphate in the formation of some aerobic biofilms, and its presence in ruminant and kangaroo saliva at similar concentrations suggests it has an important role in cellulolysis.[201] Thus elucidating the effects of saliva and its separate constituents such as phosphates is key.

More complex will be arriving at an understanding of the processes controlling the rate of digestion within biofilms. Chapter 9 hypothesises that boundary layers and internal heterogeneity mean that there is only a weak relationship between the chemistry of the menstruum and the chemistry seen by the organisms within a biofilm. It further hypothesises that it may be possible to promote the production of VFAs at the expense of growing new biomass by maintaining a low pH, and that low pH may be maintained by the combination of CO_2 and VFA. Additional species, and in particular the addition of hydrogenotrophic species such as *Methanobrevibacter*, that are believed to be among the most common rumen methanogenic species, could significantly modulate the inhibition within the film by consuming hydrogen and CO_2.[216] This could have substantial effects on local pH. Any disparity between their rate of reproduction and that of the cellulolytes would lead to significantly more complex interactions in a biofilm than have been modelled here. One objective of the research must therefore be to understand the pH environment and nutrient flows within the biofilm, and whether it is the same *in vivo* and in a reactor.

Incidentally, the role of hydrogenotrophic methanogens within a biofilm as reducers of inhibition and thus as promoters of acetogenesis appears not to have been considered in studies of methane emissions from ruminants. This is a significant area of global concern, given the contribution of ruminants to climate change by methane emissions. It is generally presumed that methanogenesis reflects loss of usable energy by the animal as well as contributing to radiative forcing. The converse may be true – hydrogenotrophic methanogenesis may, in part at least, be a promoter of rapid growth and increased animal productivity. This would be an important possible arena for research that shares with AD the need to understand rumen biofilm dynamics.

A final note on future research concerns the possibility of diverting VFA streams into products that are higher value than methane. If hydrolysis to VFA can be accelerated substantially, the opportunity exists to use the VFAs as a low cost chemical platform for longer chain and higher value products. If these could be produced biologically, with novel fast growing organisms supplanting the role of methanogens, some of the complexities of AD, including membrane filtration to remove VFAs, may be avoided and the process cost reduced even further.

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ANNEXES

ANNEX 1 – PSEUDO-CODE FOR BIOFILM MODEL

This section contains a brief outline of the Matlab model used in Chapter 10. The detailed pseudo-code used to specify the model is provided in the Supplementary Information disk.

PURPOSE

The purpose of the model is simply to help illustrate how different parameters might affect cellulolysis, and the influence of modifying those parameters. The aim is not to be a quantitative model but a model to show general principles and determine, if possible, the relative importance of factors that might be materially different between a ruminant and an AD reactor.

BACKGROUND

The model will model the growth and activity of bacterial film on a particle of insoluble substrate, and how that can be managed by increasing surface area, killing bacteria, changing how they attach and detach from the surface, etc.

The real world (as simplified for the model) looks as follows:

- A piece of biomass has two key components – lignin and cellulose.
- Cellulose is bacterial food. However it is insoluble, so bacteria have to settle on the cellulose, express a glue to stick to it, make some changes to the way their genome functions, and then start expressing enzymes that digest the cellulose.
- Thus bacteria exist in two forms – planktonic, and sessile.
    - The planktonic form have no access to food – so are probably simply floating around waiting to land on a suitable bit of cellulose.
    - The sessile form eat and grow and then reproduce.
    - Daughter bacteria adhere to the substrate in the same cell, or aa cell adjacent to the parent.

- Lignin forms in fibres, and is both indigestible by bacteria and adsorbs and inactivates the enzymes bacteria use to digest cellulose. Lignin therefore possibly acts as a barrier to the spread of bacteria on a surface.
- Bacteria age, so each time a bacterium buds off a daughter cell its activity becomes less – until eventually it probably dies.
- Diffusion. Each bacterium produces VFAs that inhibit its activity if the VFA cannot be removed. Model diffusion allows VFAs to diffuse into cells where the concentration of cellulose is less than 100% (i.e. menstruum or part digested) with the rate of diffusion between adjacent cells proportional to concentration differences. The level of VFA affects both the cellulolytic activity of the bacterium, and also the efficiency with which it store energy and thus reproduces, as opposed to using the stored energy for VFA transport across bacterial cell walls.

MODEL CHARACTERISTICS

The model has three components, bacteria, space and inhibitor concentration, each represented by a separate array. Model time is governed by a clock cycle (t) incrementing by one each cycle.

The cellulose is a sheet several units deep, and interspersed with lignin strands which are indigestible to the bacteria.

Bacteria digest cellulose in the model. Each time step a bacterium that is fixed to cellulose will carry out one or less units of activity depending how much cellulose they have left to feed on, and how old they are (measured by the cumulative number of daughters). They will produce 1 unit of inhibitor for every unit of activity.

In the original version of the model (referred to as V20) each unit of cellulose digestion resulted in an increase in 1 unit of energy retention by the bacterium. Once this figure had reached CD, a new daughter was born and the energy store reset. In V20a, the energy retained by the bacterium per unit of cellulose digestion is reduced as a function of the local inhibitor concentration – to reflect reduced efficiency of VFA transport out of the cell.

The number of units of activity (CA) needed to digest all the cellulose at a site is a key parameter because this sets the temporal resolution of a time step. High CA means many time steps to digest each site – so fine resolution. CA of 1 is a very coarse resolution.

Each time step every active bacterium will convert some cellulose into inhibitor. This inhibitor then diffuses away as defined by the Diffuse subroutine. A maximum level of inhibitor is determined at a variable distance from the cellulose/liquid interface as it is assumed that beyond this distance boundary layer effects are overtaken by turbulent flow which homogenises the liquid and VFA concentration.

A bacterium either expresses cellulolytic enzymes or produces an outgrowth (cellulosome) that reaches out to hydrolyse cellulose. In either case, a bacterium is much smaller than the volume of cellulose it can reach. In the model, substrate is represented by discrete cubical cells, each containing only either lignin or cellulose. A cell can, however be occupied by several bacteria.

Bacteria move from liquid to the substrate by settling and adhering on it. Daughters adhere to the substrate. Any bacterium that settles on a lignin cell remains there – but becomes inactive unless it is displaced later. This, in a rather imperfect way, reflects the de-activation of enzymes by lignin effectively, reducing the activity of any bacteria nearby.

Bacteria can be returned to the menstruum either by consuming al the food at their site, and having all sites above them vacant too, or by rumination. In both cases detachment is probabilistic, determined by a user input variable.

MODEL WIDE VARIABLES

1. Dimensions of initial particle is L,W,D. L and W represent the particle surface, and D the particle thickness. D must be a power of 2.
2. T is maximum number of time steps for the model
3. MINSUBST is the level of cellulose as a percentage of the initial amount, at which the model will stop – to prevent endless running to get the last molecule
4. MAXSUBST is the total amount of cellulose in the model at the start (calculated)
5. t is the current time step.

6. TR is the number of time steps between creation of a set of reporting arrays
7. LSR is the ratio of liquid to solids in the reactor.
8. ZL is the notional thickness of the liquid layer above the particle (calculated).
9. LP is the percentage of lignin
10. LL is the mean length of a lignin chain
11. NUMBACT is the cumulative number of bacteria created.
12. AR is the ratio of activity of a bacterium after each new daughter cell is budded divided by activity before each daughter. Thus AR=95% would mean activity after each daughter is 5% reduced. After 5 daughters Activity = 0.95^5 for the cell.
13. DD is the number of daughters a cell can produce before dying.
14. CA is a constant for the model, and represents the number of Activity units needed to digest 1 unit of cellulose. This should be set high enough that a bacterium can produce several daughters per unit of cellulose digested, i.e. $CA/CD \gg 1$
15. CD is the number of stored energy units (CS) a bacterium needs to produce a new daughter. 1 Activity Unit of digestion contributes 1 unit of CS.
16. A variable that must be accommodated is the function (fvin) to convert V, the concentration of inhibitors, to I, the degree of inhibition (IN). IN = fvin(V). $0 \leq IN(x,y,z) \leq 1$, and Actual Activity = Theoretical Activity * (1-IN)
17. Several different functions (fvin) need to be set up and chosen by simply entering a variable or choosing from a drop-down of some form. This will allow different functions and relationships to be explored.
18. DBL is the nominal depth of the boundary layer beyond which all inhibitor concentrations are homogenised
19. DIFFCON is the diffusion constant that sets how much of the excess V in a site moves to the adjacent site in a time step.
20. DIFFN is the number of diffusion steps per time step
21. VBG is the background level of V which is maintained at the distance DBL + 1 from the particle surface.
22. PS is the probability on any clock cycle of a given planktonic bacterium that meets the surface being in an orientation or site suitable for settling.

23. PG is the probability of a bacterium being grazed by protozoon each time step.
24. PD is the probability of detachment of an eligible bacterium on any time step.
25. RUMINATEN is the number of rumination events to model. -1 implies no limit.
26. RUMINATEF is the number of time steps between each rumination event.
27. RUMINATESA is the switch to turn surface area increase on or off in rumination
28. RUMINATEV is the switch to turn the reset of V on or off each rumination event.
29. RUMINATED is the switch to turn DETACH on or off each rumination event.
30. PRD is the proportion of active cells detached during rumination in addition to the inactive cells.
31. RUMINATEC is the rumination counter – initially zero then incremented

Items in bold are key variables that have to be easy for the user to change.

L,W are treated as the boundaries of a sample of an infinite sheet, so there are no edge effects.

The number of daughters born is a proxy for cumulative metabolic activity, and thus total accumulated damage in the cell. Each bacterium is born with the ability to perform one activity unit per time step. This decays as a function of the number of daughters as in 9 above.

One unit of activity reduces cellulose in the particle by CA-1 units (CA is always ≥1) and increases CS by 1.

Cells can die either because they reach the maximum number of daughters, or because of grazing by protozoa – which will remove random bacteria from the film, or because of lysis during rumination – either because of mechanical destruction or pH effects.

Every settled bacterium has a co-ordinate set that determines it's position in the lignocellulose matrix. When a bacterium dies it is given co-ordinates of -1,0,0 reserved for dead bacteria.

ANNEX 2 – CROSS SECTIONS AND DRAWINGS FOR NEW EXPERIMENTAL REACTOR

The following pages show a series of cross sections through a fully assembled reactor – equipped with bottom and top stirrers, and a central rotor and stator to provide shearing.

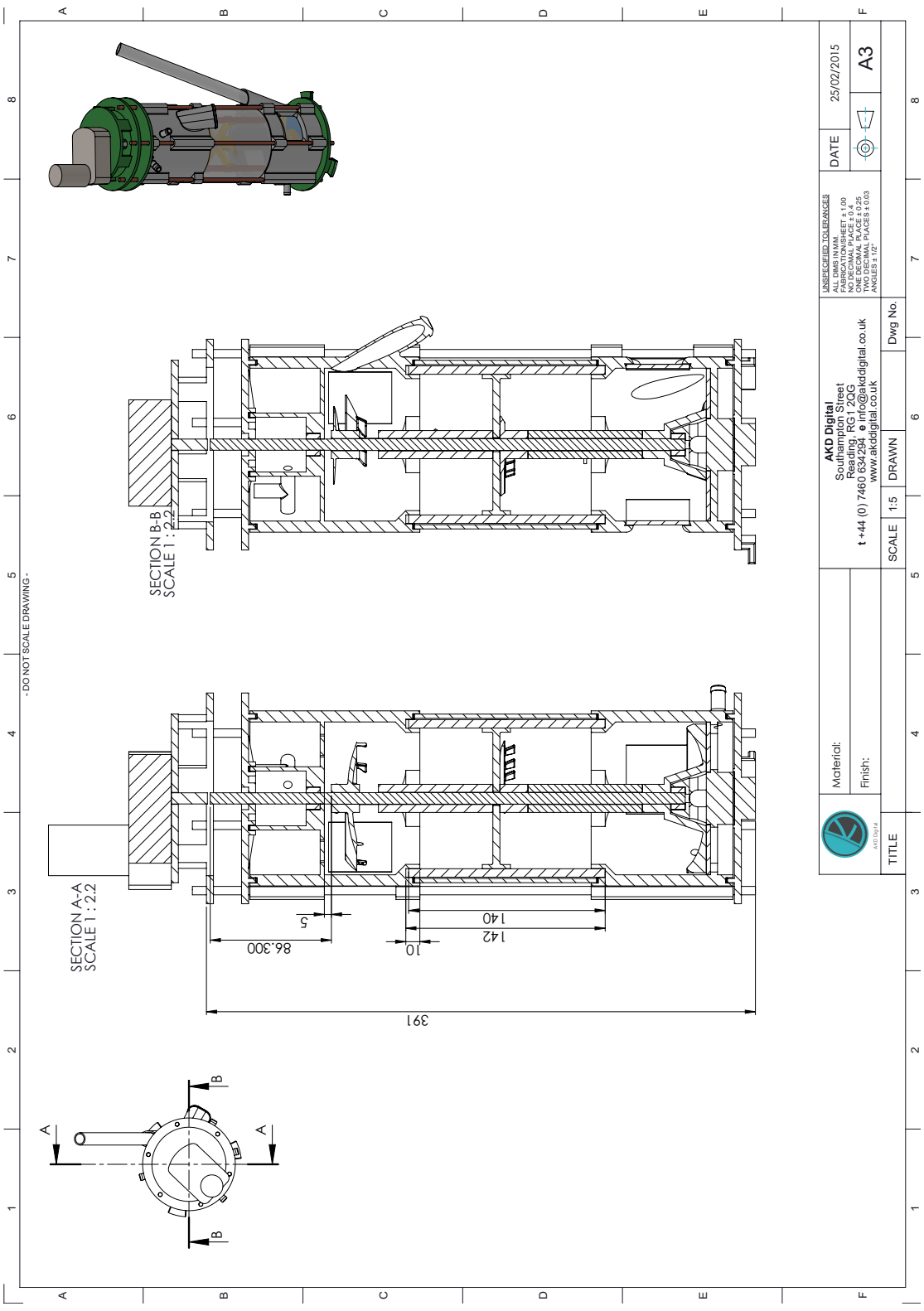

Figure 58 – Vertical sections through the assembled new experimental reactor.

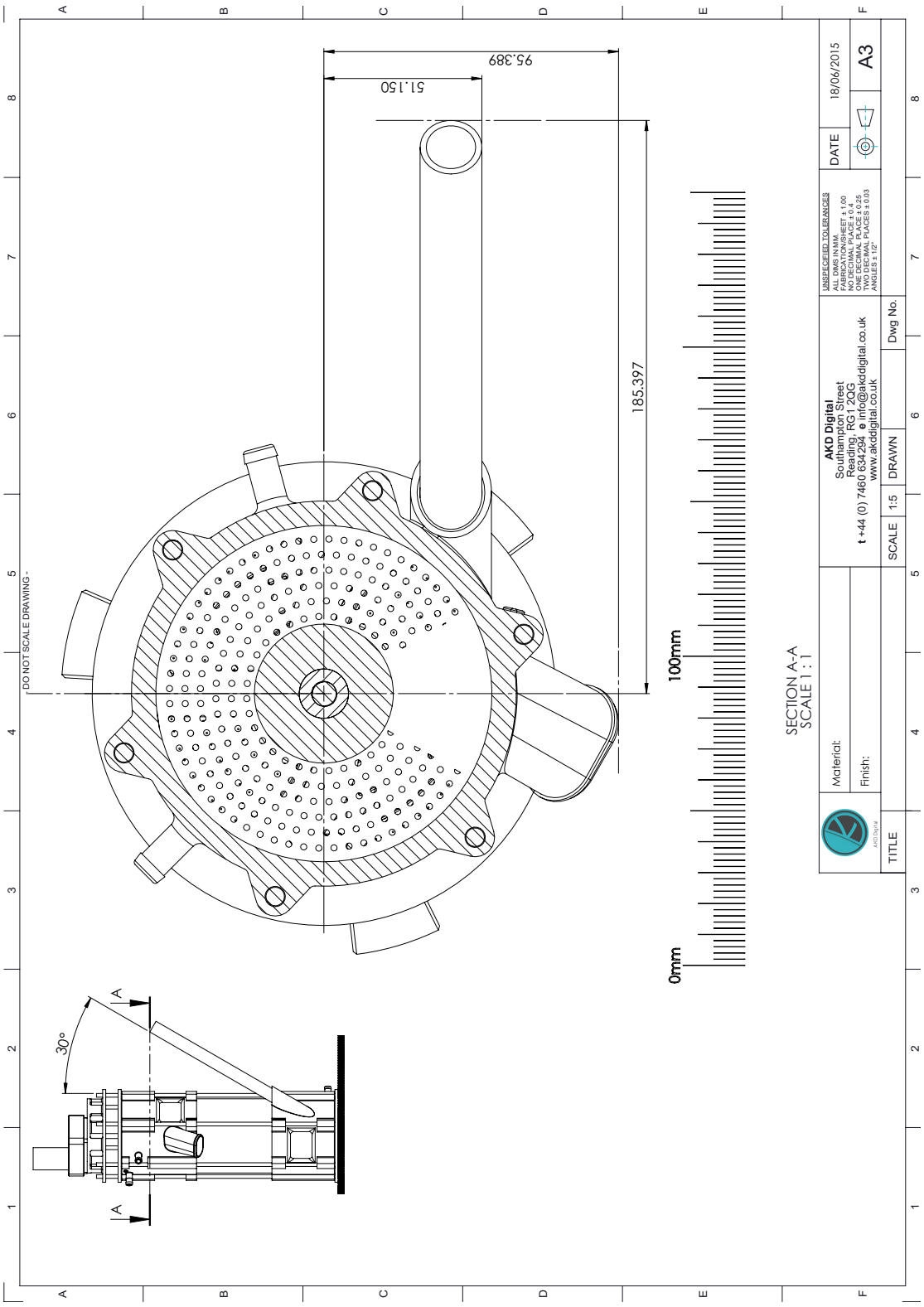

Figure 59 – Horizontal section through upper 3-d printed component.

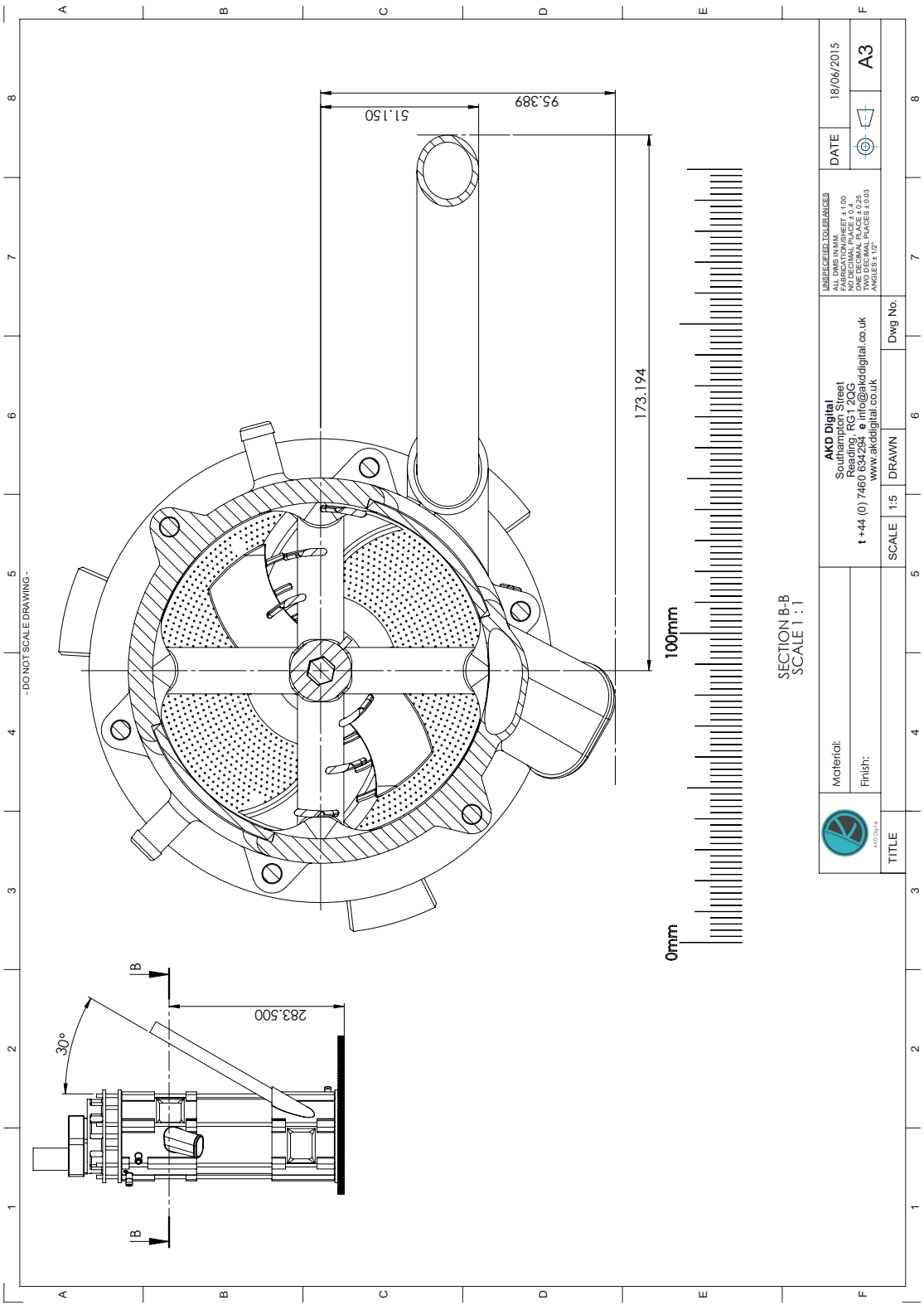

Figure 60 – Horizontal section through the upper 3-d printed component showing viewing windows, the exit tube.

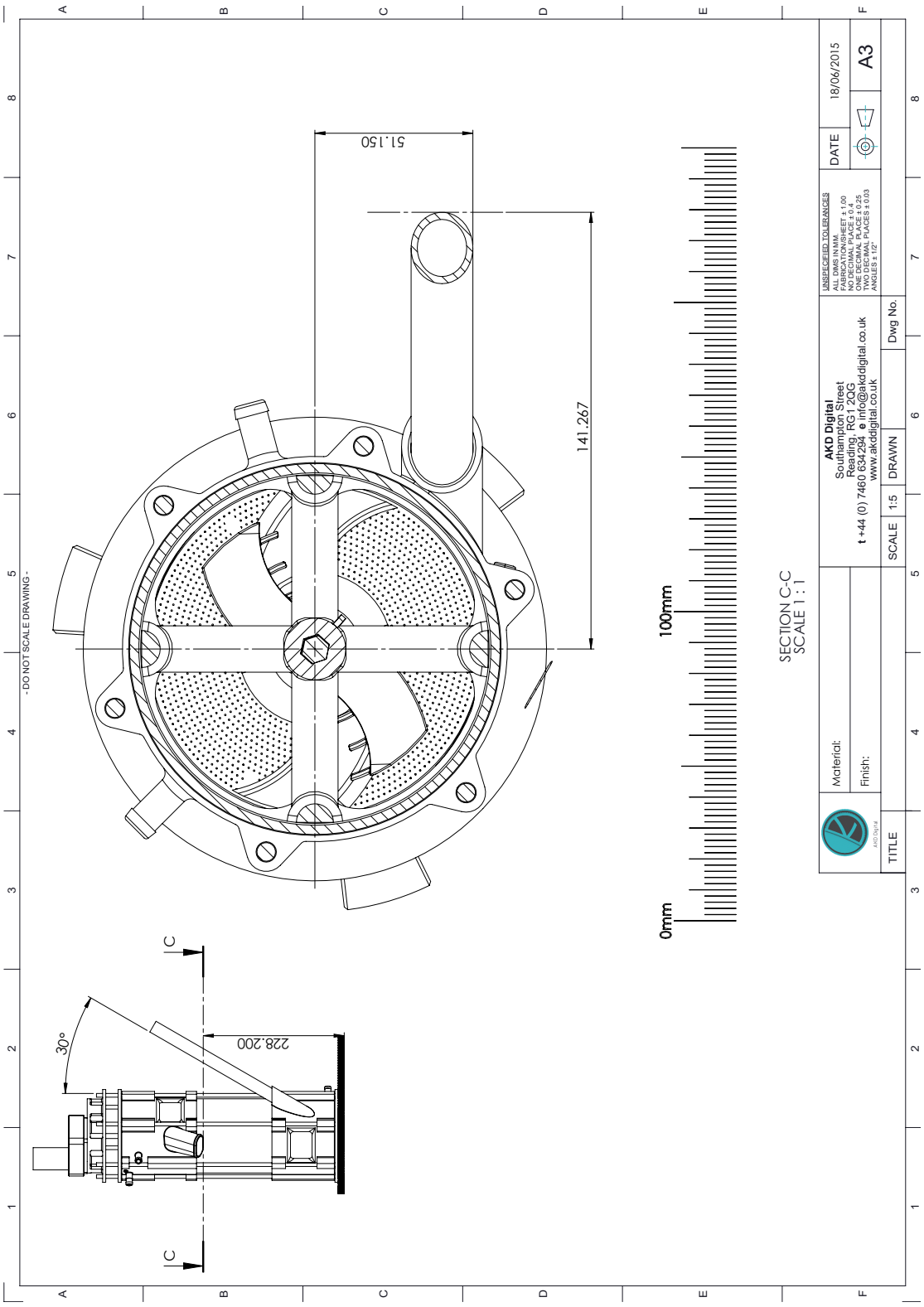

Figure 61 – Horizontal section through the upper part of the acrylic reactor tube showing a typical arrangement of internal tools – one fixed to the D shaped vertical bars with a circular hole in the central boss to allow it to be fixed, and the other (the swept arm rotor) with a hexagonal hole in the central boss to enable it to be driven.