Interlinkages of recent research outcomes for the treatment of organic wastes

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ABSTRACT. Recent research into conversion of organic wastes at Murdoch University is reviewed to highlight interlinkages, insights gained and potential future research. Central to the research underpinning the success of the one vessel sequential aerobic (to bring temperature to thermopilic)-anaerobic (to extract methane)-aerobic (to produce compost) thermophilic process is utilising the concept of electron transfer, which links aerobic and anaerobic phases through electrons as a common currency. This concept is consistent with our ability to produce electricity from the process liquor using a microbial fuel cell. Management of the liquor has been found to be critical as it is a carrier of micro-organisms that perform all the biological processes in the reactor and assist with start up. Start up has also been facilitated by the presence of grass clippings in MSW. Linkages to nitrogen removal, unusual properties of liquor, grass clippings and possible pre-treatment of cellulose wastes using fungus are highlighted.

Keywords: Organic waste; thermophilic; aerobic; anaerobic; electron transfer; process liquor; nitrogen removal; cellulose

Introduction

Treatment of organic wastes is essentially the conversion of carbon in the biodegradable fraction of the waste under controlled conditions. Otherwise the biodegradable fraction will be decomposed anyway by microorganisms under uncontrolled conditions in the environment into which the wastes are disposed. This uncontrolled degradation takes up oxygen from the disposal environment. Once oxygen is depleted degradation continues under anaerobic conditions emitting odorous gases as well as greenhouse gas methane.

Engineered treatment aims to make the process more efficient and cost effective. Much progress has been achieved in the treatment of organic wastes through research effort. Historically engineers have regarded treatment under either aerobic or anaerobic conditions (composting and anaerobic digestion respectively) in an empirical way. Provided that suitable conditions are provided microorganisms would carry out the conversion reactions. Overall conversion is empirically represented by

(i) Conversion under *aerobic conditions*:

$$C_aH_bO_cN_d + eO_2 \rightarrow nC_wH_xO_yN_z + sCO_2 + rH_2O + tNH_3 \quad (1)$$

where $C_aH_bO_cN_d$ and $C_wH_xO_yN_z$ represent on an empirical basis the composition of the organics initially and at the conclusion of the process. If all of the substrate is consumed by the microorganisms, $C_wH_xO_yN_z$ would represent the composition of the microorganisms. The coefficients of the reaction can be obtained in the usual manner by balancing the quantities of elements on the LHS and RHS of the reaction equation: $t = d – nz$, $r = 0.5 (b - nx - 3t)$, $s = a – nw$, $e = 0.5 (ny+ 2s + r - c)$

(ii) Similarly conversion under *anaerobic conditions*:

$$C_aH_bO_cN_d \rightarrow nC_wH_xO_yN_z + mCH_4 + sCO_2 + rH_2O + tNH_3 \quad (2)$$

The rate of decomposition of organic waste by microorganisms has been estimated by simple first rate kinetics.

The rate of growth of microorganisms (dX/dt) can be expressed as

$$dX/dt = (\mu - k_d)\, X \quad (3)$$

where X= microorganism concentration, kg/m^3

t= time, seconds (s)

μ= specific growth rate, s^{-1}

k_d= decay rate, s^{-1}

The specific growth rate can be related to substrate concentration by Monod equation.

The kinetic parameters are determined using simple batch tests in the laboratory where the organic waste is contacted with microorganisms. The reduction in the amount organic waste (S) and the increase in the biomass of or microorganisms (X) are monitored. The curve of S and X as a function of time is commonly called the 'growth curve'.

Applied researchers looked into how efficiency of biodegradation can be improved by experimentally conducting studies with varying environmental conditions of temperature, pH, carbon to nitrogen ratio, organic loading rate and ratio of recycled biomass to feed, while engineers and practitioners varied bioreactor configuration to achieve complete mixing, plug flow or stationary beds. Optimum conditions for composting by simple windrowing to minimise energy cost of aeration were found in this way. Similarly optimum conditions for anaerobic digestion were empirically determined.

Microorganisms and electron transfer

Consortia of bacteria were assumed to proliferate under given operating or environmental conditions. Implicit in this assumption is that suitable and appropriate bacteria are ubiquitous in nature and hence in the organic waste. Their population declines or grows depending on the surrounding environment including competition between species of bacteria. An auxiliary to this assumption is that there is no need to necessarily inoculate the organic waste, unless rapid start up is desired. Application or practice based on these assumptions is straightforward in the case of aerobic decomposition or composting. Energy may, however, be required to supply air and there is lost opportunity for extracting energy from the waste in the form of methane gas.

Start up of anaerobic digestion could be particularly difficult and failure was common. A closer look at the biochemical processes in anaerobic digestion showed that the organic materials are broken down into volatile fatty acids by specific groups of bacteria (acidogens) and that the acids are then converted to methane by separate groups of bacteria (methanogens). The different types of bacteria and their roles in the degradation organic materials and the differing environmental conditions for their optimum performance are now well appreciated. Consequently attempts at improving performance efficiency and reactor configuration have become more successful through better understanding of the existence of different groups of bacteria and their specific roles and required environmental conditions. Advances in molecular biology techniques have assisted the identification of the bacterial species.

Equations such as equations (1) and (2) represent what was considered overall stoichiometric relationship between organic substrate and their biodegradation products. Chemically the conversion was under oxidative conditions for aerobic conditions, and under reductive conditions for anaerobic conditions. It was recognised that electron transfer took place and oxygen was an electron acceptor. The organic substrate was, however, not explicitly stated as an electron donor. The recognition of the latter provides a unifying way of looking at both conversion under aerobic and anaerobic conditions. Under anaerobic conditions the organic substrate functions both as electron donor and electron acceptor. The amount of electron transferred can be worked out from the oxidation - reduction half reaction for oxygen (under aerobic conditions) or methane (under anaerobic conditions)

$$O_2 + 4 H^+ + 4e \rightarrow 2 H_2O \quad (4)$$

$$CH_4 + 2 H_2O \rightarrow CO_2 + 8 H^+ + 8e \quad (5)$$

The consumption of 1 mole of oxygen therefore involves the transfer of 4 moles of electrons, similarly the production of 1 mole of methane involves the transfer of 8 moles of electrons.

Viewed as electron equivalents, the electrons can be regarded as the currency for bioconversion irrespective of environmental conditions. Bacteria have been discovered that can transfer the electrons to an electrode as electron acceptor and hence electricity can be produced. Electron mediators in solution may also assist in the shuttling of electrons.

Though carbon has been a major concern for proper treatment of organic waste, the importance of nitrogen cannot be overlooked particularly with wastes containing high protein content such as food wastes. Because of the C:N of bacterial biomass an optimum ratio is required for rapid conversion of substrate to biomass with too high a ratio slowing down the conversion. In addition a by-product of conversion of high C:N substrate is leachate and/ or gas containing high ammonia.

In this paper we highlight recent research in our laboratories into the above topics and share our insight from the interlinkages of research outcomes. Detailed description of the each of the research projects is referenced to our published papers.

Case studies

Carbon reduction

Our research experience has centred on developing an in-vessel aerobic – anaerobic – aerobic sequential treatment of the organic fraction of municipal solid waste (OFMSW) to extract methane under thermophilic conditions and produce stabilised product (compost/ soil conditioner) [1-4]. What we have learnt from laboratory experiments is that thermophilic anaerobic digestion is more rapid than aerobic treatment. Figure 1 shows electron flux during a sequence or aerobic – anaerobic – aerobic treatment process calculated using equations (10) and (11). Using electron equivalents enables aerobic and anaerobic processes to be directly compared. It may be counter-intuitive to the usual comparison between composting and anaerobic digestion, but the self-insulating thermal property of OFMSW can bring temperatures to above the optimum thermophilic range for composting and reaction rate decreases.

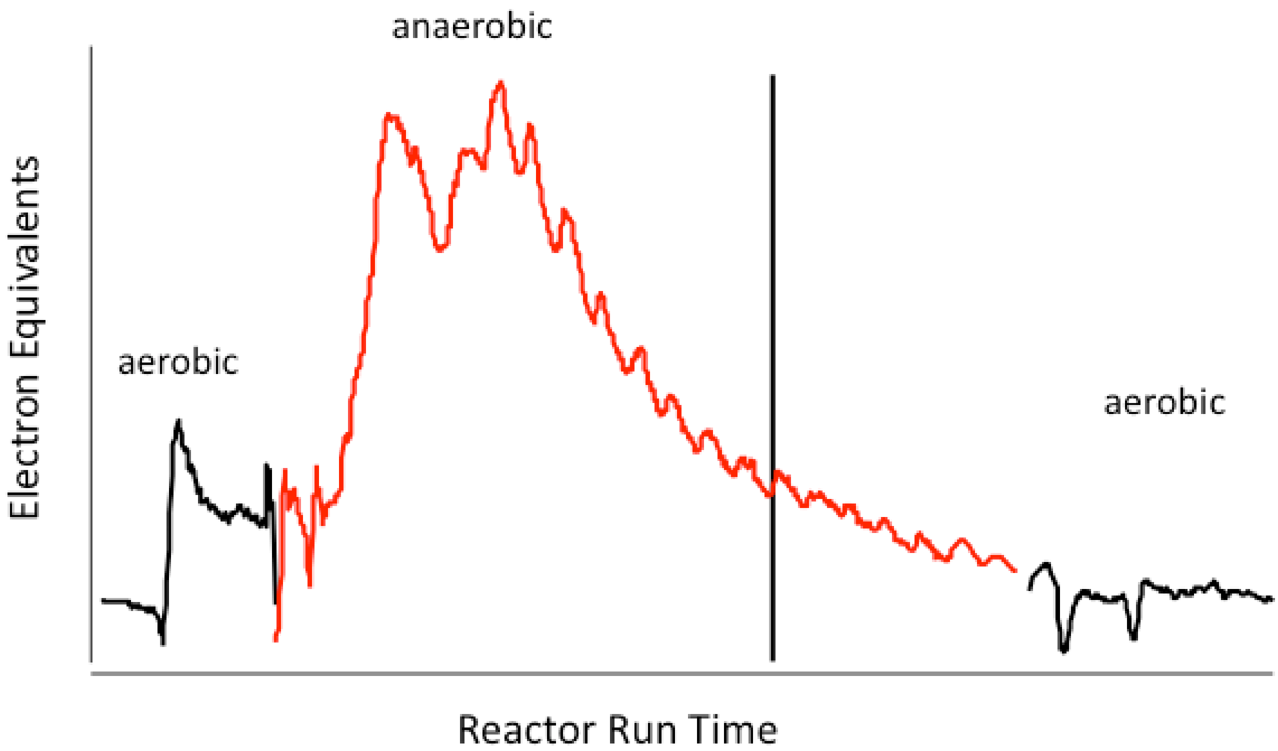

Figure 1. Electron flux during in-vessel treatment of OFMSW; reactor run time is 3 weeks.

Figure 1 demonstrates that the rate of electron transfer in the batch reaction largely follows the empirical equations (3) to (8).

The sequence of aerobic and anaerobic treatments and the apparent ease of bringing the anaerobic stage to optimum conditions demonstrates that there are anaerobes both acidogens and methanogens that tolerate oxygen and widely differing oxidation-reduction potentials. The conditions achieved in the laboratory are replicated in full scale application [5]. The electron flux during the initial aerobic stage in full scale operation is less than in the laboratory, because the self weight of the waste squeezes water out and hydrolysis may be limited.

Start up of full scale thermophilic anaerobic digestion can be problematic because of lack of readily available inoculum in quantity sufficient for large scale operation. We have found, to our surprise, that OFMSW in Perth contains within it the required inoculum [6-8]. Looking closely into which component of OFMSW carries the methanogens, it is likely the turf faction harbours the micro-organisms. This again demonstrates the resilience of the methanogens to exposure to oxygen.

Excess liquor generated during the anaerobic stage of a full scale operation is recycled to help with maintaining high populations of micro-organisms in the vessel, and can be an inoculum for the next batch of feed or for a new reactor.

Nitrogen reduction or recovery

Anaerobic digestion of OFMSW generates ammonia from the breakdown of proteins in food waste. There is a benefit from the production of ammonia as it is alkaline and buffers the production of acids in the first stage of digestion. Addition of alkalinity is hence not necessary to control pH to allow methangens to thrive. On the other hand too high ammonia concentration, particularly in molecular form, is toxic to methanogens and to be avoided.

Ammonia release into the gas phase can also be a problem. We have developed a biofilter that can remove the ammonia and convert it to nitrogen gas [9]. This is made possible by developing a gradient of concentration in the biofilter by returning condensate, from the cooled gas outlet, into the top of the filter. As the water moves down the filter it dissolves any soluble materials and brings them down to the bottom and concentrating them there.

Incoming gas containing ammonia is adsorbed by the biofilm of bacteria and oxidised to nitrite. Build up of nitrite can be inhibiting to continuing bacterial ammonia oxidation, but because it is washed down product inhibition is avoided. Accumulated nitrite at the bottom of the biofilter encounters incoming ammonia, and because of the high concentrations of ammonia and nitrite chemical reaction as shown in Figure 2 takes place. This reaction is catalysed by zeolite that is used as the matrix for the biofilter. The zeolite additionally acts as a buffer because it is a cation exchanger and adsorbs ammonia during any episode of high ammonia concentration. A novel aspect of the design of the biofilter is that the return of condensate is controlled so that only moisture that is evaporated from the biofilter is returned, a net result is no leachate is generated.

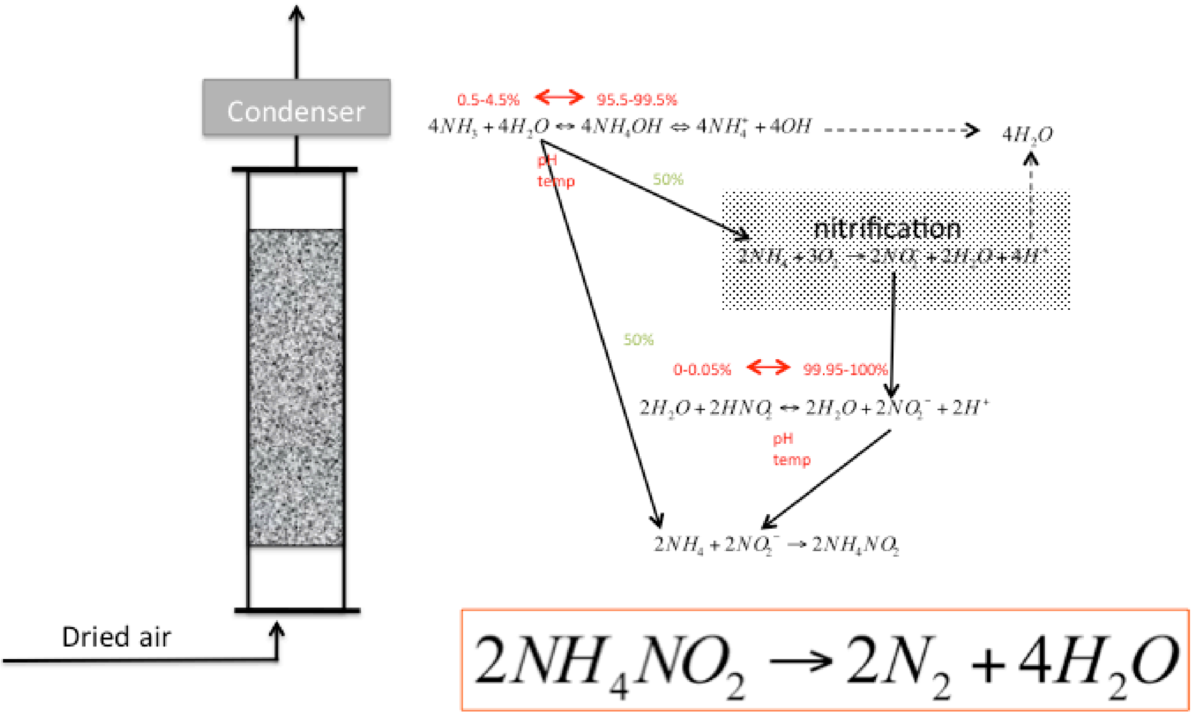

Figure 2. Schematic diagram of a biofilter with condensate return establishing a concentration gradient

down the filter showing chemical reactions that removes ammonia from the incoming gas and converting it to nitrogen gas.

As mentioned above ammonia build up in the recycled liquor during the anaerobic digestion stage should be controlled to prevent inhibition of methanogenesis. Precipitation of ammonia and phosphate as struvite ($MgNH_4PO_4.6H_2O$) produces a slow release fertiliser [10]. Addition magnesium is required as shown by equation [12] and addition of phosphate may be required depending on the concentration of ammonia and phosphate and how much ammonia is to be removed. Leaving behind some ammonia is desirable as an alkalinity buffer.

$$NH_4^+ + Mg_2^+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_4.6H_2O \quad (12)$$

Removal of ammonia in a low carbon to nitrogen ratio environment is feasible with parallel nitrification and denitrification process developed in our laboratory [11]. Organic carbon in the liquor is adsorbed into a biofilm in a fixed bed and the ammonia rich liquor is then passed through a nitrifying fixed bed biofilm to be nitrified under aerobic conditions. The nitrified liquor is then passed through a parallel fixed bed that had adsorbed organic carbon in the previous cycle to be denitrified under anoxic conditions.

A microbial fuel cell can be used to recover ammonia from ammonia rich liquor [12, 13]. Bacteria in the anode chamber consuming organic carbon donate electrons to the anode. Electricity is generated when the electrons flow through an external circuit to the cathode. An equivalent amount of protons needs to migrate to from the anode chamber to the cathode chamber through a membrane separating the two chambers. The concentration of protons is however small relative to the concentration of ammonium, hence ammonium migrates to the cathode chamber and is concentrated there. Recovery as ammonia gas or as struvite is feasible.

Observation of properties of anaerobic liquor

The liquor from the anaerobic digestion stage has two properties that we have unexpectedly found to be unusual. One is its ability to dechlorinate chlorinated organic compounds such as hexa-chloro-butadiene (HCBD). HCBD has been shown to be dechlorinated when a suitable electron mediator such as cyanocobalamin (vitamin B12) is present [14]. This implies that the liquor similarly contains suitable mediators. The mediators in the liquor are yet to be specifically identified.

The liquor (raw or sterilised) is also able to suppress the growth of plant pathogen *Phytophthora cinammomi* in culture media as well as in pot trials of seedlings affected by the fungus [15]. Again the compounds that are antagonistic to the pathogen have not been fully identified.

Further carbon reduction

The compost byproduct of the aerobic-anaerobic-aerobic sequential treatment of OFMSW is still relatively rich in organic materials dominated by cellulose. The latter is difficult to degrade under anaerobic or aerobic conditions over a short period of time (3 weeks). Enzymatic treatment using cellulase produced in situ has been shown in our laboratory to be feasible and thus avoiding the purchase and use of commercially produced enzyme [16 -18]. The cellulose substrate is inoculated with materials containing *Trichoderma reesei* and cellulase is produced in-situ during solid state fermentation. Once the cycle production has been established 5% of the treated substrate can be recycled as inoculum. Strict sterile conditions have been found to be unnecessary with the process requiring only techniques used for home brewing. The sugars produced can be further fermented to ethanol or converted to methane by anaerobic

Discussion

Interlinkages

Microorganisms and their roles

Centred on carbon and nitrogen reduction our research aims to improve the processing of the OFMSW. What became clear to us is the importance of microorganisms and their roles. Though this is obvious because we use micro-organisms, our understanding of different groups of bacteria performing different roles particularly in anaerobic digestion has enabled us to configure the processes to provide optimum conditions for each group and to sequence the processes appropriately. Much of our understanding has been derived from research into the treatment of wastewater and wastewater sludge, but the same processes apply to the treatment of MSW.

Molecular biology techniques have enabled us determine the species of microorganism performing specific roles. For improving treatment processes it is the roles or functions of the different groups of microorganism that are important. The conditions for optimum functioning of each specific group of microorganisms can then optimised even if we do not know specific species within the group. The long held assumption that given the right conditions the right or appropriate microorganisms will grow and multiply to perform their roles appears to still hold.

For anaerobic digestion we can configure a reactor to provide each function (hydrolysis, acidogenesis, methanogenesis) a separate chamber and optimise operating conditions in each. This could work well or make the system too complex. It can be considered surprising that a one chamber anaerobic digestor can work well and achieve the desired objective of producing energy (methane) and compost/ soil conditioner. It can be argued that the relatively long residence time of the substrate in the reactor caters for the slowest growing microorganisms and this may well be the case.

The one vessel anaerobic digestion process appears not to suffer from imbalance between carbon and nitrogen provided that the recycled liquor is monitored and controlled so that ammonia does not accumulate to excessive or toxic levels. Understanding the microorganisms that perform nitrification and denitrification has enabled us to remove excess ammonia as nitrogen gas. Recovery of ammonia is also possible by our knowledge of its chemistry.

Electron transfer

Composting and anaerobic digestion are oxidation and reduction processes and overall these involve, in the biochemical sense, net transfers of electrons. Looking at these processes and their sub-processes in this way enables us to quantify amount of organic materials oxidised or reduced on the same basis of electron flux. The rate of transfer can similarly characterise the rate of reaction so that both composting and anaerobic digestion can be compared directly. The fact that some microorganisms can donate their electrons to an electrode (anode) or receive electrons from an electrode (cathode) is the basis of a microbial fuel cell. Though the amount of electricity generated is comparable to the energy in methane produced, the complexity of stacks of microbial fuel cells has not made it economically viable. Such a system may be used, however, not solely to produce electricity but also, for example, to recover ammonia.

The transfer of electrons in a microbial community involves electron mediators (e.g. compounds such as vitamin B) or bacteria that have extended filaments as conduit to transfer electrons. As our understanding of these improve, we may be able to exploit it for specific purposes.

Much attention of microorganisms for composting and digestion has been on bacteria. Fungi have been known to decompose cellulose and we could make use of these and others as pre-treatment or post-treatment. The latter is the case when compost is windrowed over a longer period (order of weeks rather than days).

Conclusion

Our understanding of the function of microorganisms, particularly in anaerobic digestion, has enabled us to optimise this process for carbon reduction, its recovery as methane and for nitrogen reduction by transformation to nitrogen gas or recovery as fertiliser. Appreciation of composting and anaerobic digestion as electron transfer processes has opened areas of research into the use of bioelectrochemical systems. Further advances will be facilitated by greater understanding the function of specific groups of micro-organisms (including fungi), their mechanisms of electron transfer, ability to suppress pathogens and interactions, in the case of anaerobes, with their hosts.

Acknowledgements

We highly appreciative the contribution of staff and PhD students in our research group to our increased understanding of the topics in this paper through their exploratory research questions and ideas. We would like to mention in particular Senior Research Fellow Wipa Charles, PhD candidates and graduates Lee Walker, Suwat Suwannoppadol, Sebastian Vithzum von Eckstaedt, Leonie Hughes, Ka Yu Cheng, Raphael Flavigny, Donny James and Mitch Lever. We are grateful of the support of our industry partners through the Environmental Biotechnology Cooperative Research Centre.

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