Production of bioenergy and biochemicals from industrial and agricultural wastewater

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The building of a sustainable society will require reduction of dependency on fossil fuels and lowering of the amount of pollution that is generated. Wastewater treatment is an area in which these two goals can be addressed simultaneously. As a result, there has been a paradigm shift recently, from disposing of waste to using it. There are several biological processing strategies that produce bioenergy or biochemicals while treating industrial and agricultural wastewater, including methanogenic anaerobic digestion, biological hydrogen production, microbial fuel cells and fermentation for production of valuable products. However, there are also scientific and technical barriers to the implementation of these strategies.

The bioprocesses that will be used to treat wastewater in the future will be chosen as they have been in the past: according to technical feasibility, simplicity, economics, societal needs and political priorities. The needs and political priorities of a sustainable society, however, provide pressure that will shift the focus on wastewater treatment from pollution control to resource exploitation. Many bioprocesses can provide bioenergy or valuable chemicals while simultaneously achieving the objective of pollution control. Industrial wastewaters, for example, from food-processing industries and breweries, and agricultural wastewaters from animal confinements are ideal candidates for bioprocessing because they contain high levels of easily degradable organic material, which results in a net positive energy or economic balance, even when heating of the liquid is required. In addition, they have a high water content, which circumvents the necessity to add water. Such wastewaters are potential commodities from which bioenergy and biochemicals may be produced. Recovery of energy and valuable materials might reduce the cost of wastewater treatment, and somewhat reduce our dependence on fossil fuels.

This review describes four different bioprocessing strategies that can be used to treat industrial and agricultural wastewater, with the generation of valuable products. Three of these bioprocessing strategies result in the production of bioenergy (methane, hydrogen, electricity), and the fourth processing strategy produces valuable biochemicals by fermentation. Although phototrophic processes can also be used to produce hydrogen and valuable products, this review focuses exclusively on chemotrophic (i.e. dark) processes; in particular, on recent technological developments, but barriers to implementation and unresolved scientific questions will also be discussed. For each of the bioprocessing strategies, we will consider whether the technology is ready for full-scale implementation, whether the products can be easily separated from the treated wastewater, whether mixed, pure or well-defined co-cultures are preferable, and whether the products have sufficient value to justify the added complexity over conventional wastewater treatment processes.

Biological methane production from organic material in industrial and agricultural wastewater

Methanogenic anaerobic digestion of organic material in wastewater (Table 1, reaction 11) has been performed for about a century and is advantageous over aerobic active sludge systems because of its high organic removal rates, low energy-input requirement, energy production (i.e. methane), and low sludge production. The food web of anaerobic digestion is reasonably well understood (Box 1). An important breakthrough was made ~30 years ago, with the development of the upflow anaerobic sludge blanket [UASB] reactor [1], which efficiently retains the complex microbial consortium without the need for immobilization on a carrier material (for example, as a biofilm) by formation of biological granules (i.e. granulation; self-immobilization) with good settling characteristics. The mean cell residence time, that is, the average time a typical microbial cell remains in the reactor, of UASBs is much longer than the hydraulic residence time (the average time the wastewater remains in the reactor), due to this self-immobilization process. Performance depends on the mean cell residence time and reactor

Table 1. Reactions for the different (bio) process strategies

Biotic or abiotic process	Reaction	Number (letter, if shown, is that in Figure I in Box 1)
Hydrogen fermentation to acetic acid	$C_6H_{12}O_6 + 2H_2O \leftrightarrows 4H_2 + 2CH_3COOH + 2CO_2$	1 (a)
Hydrogen fermentation to butyric acid	$C_6H_{12}O_6 \leftrightarrows 2H_2 + CH_3CH_2CH_2COOH + 2CO_2$	2 (a)
Fermentation to ethanol	$C_6H_{12}O_6 \leftrightarrows 2CH_3CH_2OH + 2CO_2$	3 (a)
Propionic acid production with hydrogen	$C_6H_{12}O_6 + 2H_2 \leftrightarrows 2CH_3CH_2COOH + 2H_2O$	4 (a)
Ethanol production with hydrogen	$CH_3COOH + H_2 \leftrightarrows CH_3CH_2OH + H_2O$	5
Syntrophic propionic acid oxidation	$CH_3CH_2COOH + 2H_2O \leftrightarrows CH_3COOH + 3H_2 + CO_2$	6 (b)
Syntrophic butyric acid oxidation	$CH_3CH_2CH_2COOH + 2H_2O \leftrightarrows 2CH_3COOH + 2H_2$	7 (b)
Syntrophic acetic acid oxidation	$CH_3COOH + 2H_2O \leftrightarrows 4H_2 + 2CO_2$	8 (c)
Hydrogenotrophic methanogenesis	$4H_2 + CO_2 \leftrightarrows CH_4 + 2H_2O$	9 (d)
Acetoclastic methanogenesis	$CH_3COOH \leftrightarrows CH_4 + CO_2$	10 (e)
Methane formation from glucose	$C_6H_{12}O_6 \leftrightarrows 3CH_4 + 3CO_2$	11 (a,b,c,d,e)
Catalytic methane conversion to syngas	$CH_4 + H_2O \leftrightarrows 3H_2 + CO$	12
Catalytic gas-shift reaction	$CO + H_2O \leftrightarrows H_2 + CO_2$	13
Hydrogen fuel cell	$2H_2 + O_2 \leftrightarrows 2H_2O + electricity$	14
Methane fuel cell	$CH_4 + 2O_2 \leftrightarrows CO_2 + 2H_2O + electricity$	15
MFC	$C_6H_{12}O_6 + 6O_2 \leftrightarrows 6CO_2 + 6H_2O + electricity$	16
Cellulose bioconversion	$[-C_6H_{11}O_6-]_n + aH_2O \leftrightarrows bCH_3COOH + cCH_3CH_2OH + dCO_2 + eH_2$	17
Polyhydroxyalkanoates formation	$aC_6H_{12}O_6 + bO_2 \leftrightarrows [-COOH(CH_2)_3COO-]_n + cH_2O$	18
Dyes formation	$[-C_6H_{11}O_6-]_n + aNH_3^- + bH_2O \leftrightarrows cC_{23}H_{26}O_5 + dC_{22}H_{27}O_5 N + eC_{21}H_{22}O_5$	19

volume depends on the hydraulic residence time, therefore, UASBs can efficiently convert wastewater organic compounds into methane in small 'high-rate' reactors. Approximately 60% of the thousands of anaerobic full-scale treatment facilities worldwide are now based on the UASB design concept, treating a diverse range of industrial wastewaters [2,3].

It was originally thought that a continuous upflow-hydraulic pattern was required for granulation, but this phenomenon was recently observed in a continuously-fed horizontal-flow bioreactor that incorporated a migrating blanket within a compartmentalized reactor (a multi-vessel; anaerobic migrating blanket reactor [AMBR]) [4]. Due to the AMBR's inherent dynamic conditions, the organic removal rates are higher than those in UASB reactors [5,6].

A significant limitation of UASB reactors is the interference of suspended solids in the incoming wastewater with granulation and reactor performance [7]. Hence, other high-rate systems, such as the anaerobic

Box 1. Food web of methanogenic anaerobic digestion

Anaerobic bioconversion of complex organic material to methane requires four major steps and five physiologically distinct groups of microorganisms. Elements of the food web of methanogenic anaerobic digestion are expected to occur also for biological hydrogen production, microbial fuel cells and biochemical production. As shown in Figure I, complex organic polymers (e.g. proteins, polysaccharides) are hydrolyzed to monomers by fermentative bacteria (a), which ferment the monomers to a mixture of low-molecular-weight organic acids and alcohols. These fermentation products are further oxidized to acetic acid and hydrogen by obligatory hydrogen-producing acetogenic bacteria (b) through a process called acetogenesis. Acetogenesis also includes acetate production from hydrogen and carbon dioxide by acetogens and homoacetogens (c). Hydrogen-producing acetogenic bacteria (b) grow in syntrophic associations with hydrogenotrophic methanogens (d), which keep the hydrogen partial pressure low enough to allow acetogenesis to become thermodynamically favorable (this process is referred to as interspecies hydrogen transfer) [58]. Finally, acetoclastic methanogens (e) convert the acetate to methane and carbon dioxide (methanogenesis). Although ~70% of methane produced in many natural and engineered systems is due to acetoclastic methanogens, it is increasingly clear that many stressed and thermophilic systems use an alternative pathway: syntrophic oxidation of acetate to carbon dioxide and hydrogen by acetogenic or homoacetogenic bacteria (c) coupled to hydrogen consumption by hydrogenotrophic methanogens [8,59].
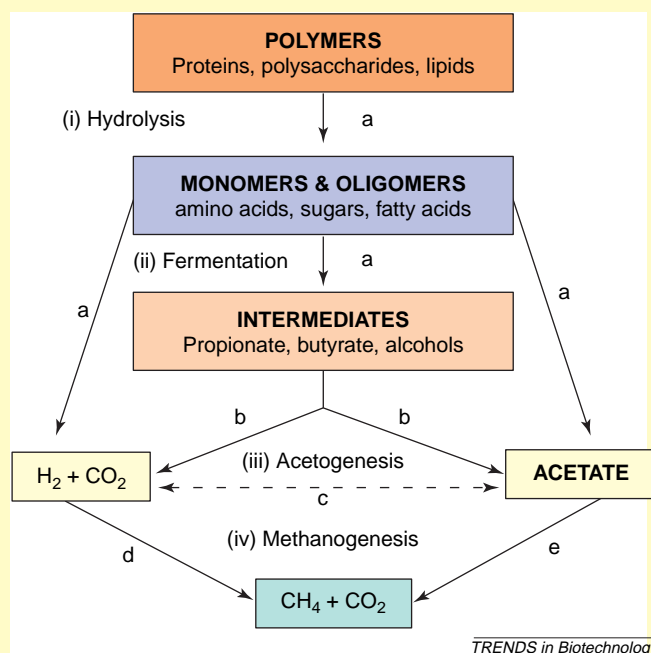

Figure I. Intricate food web of methanogenic anaerobic digestion. Several trophic groups of microorganisms work together to convert complex organic material into methane and carbon dioxide

sequencing batch reactor (ASBR), were developed to better handle high-suspended solids in wastewater. ASBRs are single-vessel bioreactors that operate in a four-step cycle: (i) wastewater is fed into the reactor with settled biomass; (ii) wastewater and biomass are mixed intermittently; (iii) biomass is settled and; (iv) effluent is withdrawn from the reactor. ASBRs are particularly useful for agricultural waste, and it has recently been scaled up for on-farm treatment of dilute swine waste [8].

The methane that is produced by anaerobic digestion has traditionally been used as a fuel source, usually, for on-site heating or electricity production. Recently, methane has also been converted to other useful products, such as methanol for use in production of biodiesel, for example, by production of syngas (a mixture of hydrogen and carbon monoxide; Table 1, reactions 12 and 13) in downstream chemical processes [9]. Production of syngas requires the removal of impurities, such as hydrogen sulfide, in the digester biogas, which can poison the catalyst. In addition, direct conversion of methane to electricity in solid-oxide fuel cells after a single-step anaerobic digester might soon become feasible (Table 1, reaction 15) [10].

Biological hydrogen production

Much recent interest has been expressed in the biological production of hydrogen from wastewater by dark fermentation, due to its potential importance in our economy [11–14]. Biological hydrogen production shares many common features with methanogenic anaerobic digestion, especially the relative ease with which the two gaseous products can be separated from the treated wastewater. The mixed communities involved in both bioprocesses share some common elements but with one important difference: successful biological hydrogen production requires inhibition of hydrogen-using microorganisms, such as homoacetogens (see Figure I in Box 1, group c) and methanogens (see Figure I in Box 1, group d). Inhibition is commonly accomplished by heat treatment of the inoculum to kill all microorganisms except for spore-forming fermenting bacteria (for example, species from the families *Clostridiaceae*, *Streptococcaceae*, *Sporolactobacillaceae*, *Lachnospiraceae*, and *Thermoanaerobacteriacea* [15–17]) (see Figure Ia in Box 1). Other methods that have been used include the operation of reactors at high dilution rates [18] or low pH [19].

Considerable effort has been devoted to optimizing operational environmental conditions to maximize hydrogen production (examples of optimization efforts are given in Table 2). Conceptually, important efforts are those that prevent consumption of hydrogen by, for example, propionic acid-producing bacteria, ethanol-producing bacteria, and homoacetogens (Table 1, reaction 4, reaction 5, and the reverse of reaction 8, respectively); and those that channel more reducing equivalents towards reduction of protons by hydrogenases (see Box 2). Operating bioreactors at low hydrogen partial pressure, perhaps by sparging with nitrogen gas to strip hydrogen from the solution as fast as it is produced [19,20], accomplishes both efforts simultaneously.

Unfortunately, optimization of biohydrogen production focuses on a relatively small fraction of the total hydrogen equivalents that are present in wastewater. For example, optimization of hydrogen production from related hexoses, at best, results in production of four moles of hydrogen per mole of hexose, because two moles of acetate are also formed (Table 1, reaction 1). Complete oxidation to carbon dioxide and hydrogen, however, would produce 12 moles of hydrogen per mole hexose (reaction 1 plus reaction 8 in Table 1). Actual yields are even lower than the four moles of hydrogen that are theoretically possible, typically ranging from <1 to ~2.5 moles hydrogen per mole hexose (Table 2). When butyric acid is produced as a major fermentation product, only two moles of hydrogen can be produced (Table 1, reaction 2). Hydrogen yield is even lower when more reduced organic compounds, such as lactic acid, propionic acid, and ethanol, are produced as fermentation products, because these represent end products of metabolic pathways that bypass the major hydrogen-producing reaction in carbohydrate fermentations (Table 1, reaction 3). Thermodynamic limitations for hydrogen production are explained in Box 2.

It appears that, even under optimized conditions, one cannot expect to recover more than ~15% of the electron equivalents in a high-carbohydrate wastewater as hydrogen, thus, it is not surprising that several research groups are considering implementing two-step processes, involving biohydrogen production followed by methanogenic anaerobic digestion to increase the energy yield of the overall process [11,21]. As described previously, methanogenic anaerobic digestion is a mature, reliable technology that has been demonstrated in thousands of full-scale facilities worldwide. Catalytic conversion of methane to hydrogen gas is also a well-developed and reliable process (Table 1, reactions 12 and 13) [9]. Therefore, direct biological production of hydrogen through dark fermentation appears to be restricted to a pre-treatment step in a larger bioenergy or biochemical production concept. Another anticipated disadvantage of large-scale hydrogen production that needs to be addressed during scale-up is the escape of hydrogen through large plastic enclosures and thin metal sheets that might occur due to the high diffusivity of hydrogen.

Biological electricity production

Microbial fuel cells (MFCs) have also been suggested as an alternative to follow biohydrogen production in a two-step treatment process [22]. Many researchers, however, have successfully generated electricity biologically in a single-step process (Table 1, reaction 16) [23–26]. Figure 1 shows a generic schematic of how an MFC works. In principle, MFCs are similar to hydrogen fuel cells, which channel protons from an anode compartment to a cathode compartment through an electrolyte membrane (i.e. electronically insulated proton-exchange membrane) with electrons going in the same direction via a conductive wire. A hydrogen fuel cell oxidizes hydrogen to electrons and protons on the anode and reduces oxygen to water on the cathode (Table 1, reaction 14). Gas-permeable noble metals are used as electro-catalysts on the anode and cathode sides [27]. In an MFC, conversely, anaerobic microorganisms oxidize organic material in the anode chamber and they transfer the derived reducing

## Box 2. Physiological limitations for biological production of hydrogen

Hydrogen production from organic substrates is limited by the thermodynamics of the hydrogenase reaction, which involves the enzyme-catalyzed transfer of electrons from an intracellular electron carrier molecule to protons. Unfortunately, protons are poor electron acceptors ($E'_{H_2} = -414$ mV); so, the electron donor must be a strong reducing agent. Ferredoxin is a low-potential ($E^{o'}_{Fd} \approx -400$ mV, depending on source) iron-sulfur protein that is capable of reducing protons to hydrogen [60]. Another important intracellular electron carrier, NADH, has a higher redox potential ($E^{o'}_{NADH} = -320$ mV). The ability of reduced ferredoxin and NADH to reduce protons is determined by the redox potential of the net reaction under actual conditions. Assuming the intracellular concentrations of the oxidized and reduced forms of ferredoxin and NADH are about equal, hydrogen production becomes thermodynamically unfavorable at hydrogen partial pressures greater than:

$$P_{H_2,\max} \leq \exp\left\{\frac{2F(E^{o'}_{H_2} - E^{o'}_x)}{RT}\right\}$$

where $E^{o'}_X$ is the redox potential of the electron donor, F is Faraday's constant, R is the ideal gas constant, and T is the absolute temperature. For ferredoxin, hydrogen production can continue as long as the hydrogen partial pressure is less than ~0.3 atm (3×10^4 Pa); for NADH, the partial pressure of hydrogen must be less than ~6×10^{-4} atm (60 Pa). Note that these values assume equal concentrations of the oxidized and reduced forms of the electron donors. Higher hydrogen partial pressures can be achieved if the ratio of reduced

ferredoxin to oxidized ferredoxin is greater than one. The free energy change of the pyruvate-ferredoxin oxido reductase reaction ($\Delta G^o;' = -2.1$ kcal/mole) is sufficient to allow the reaction to proceed with more than a tenfold excess of products over reactants. Hence, the frequent observation of more than 30% hydrogen in reactor headspace is not unexpected.

In most systems for biological production of hydrogen, all of the observed hydrogen can be attributed to electrons derived from a single reaction: oxidative decarboxylation of pyruvate by pyruvate:ferredoxin oxidoreductase (Figure II). Hexoses can be metabolized to pyruvate through several pathways, often involving the Embden-Meyerhoff-Parnas (i.e. glycolysis) or the Entner-Doudoroff pathways. Both of these pathways produce two moles of pyruvate and two moles of NADH for every mole of hexose that is transformed [60]. Therefore, hexose metabolism by bacteria that contain pyruvate:ferredoxin oxidoreductase can result in formation of 2 moles of hydrogen per mole of hexose. If the hydrogen partial pressure is sufficiently low (<60 Pa), the NADH that is produced may also be used to generate hydrogen (at best, an additional 2 moles of hydrogen per mole of hexose), but most of the NADH will probably be oxidized through other fermentation pathways, such as butyrate fermentation (Figure II, step 4). Some fermentation products (e.g. ethanol and lactate) represent the operation of alternative pathways for pyruvate metabolism that compete with pyruvate:ferredoxin oxidoreductase. As such, they will usually be associated with systems that produce less than two moles of hydrogen per mole of hexose.
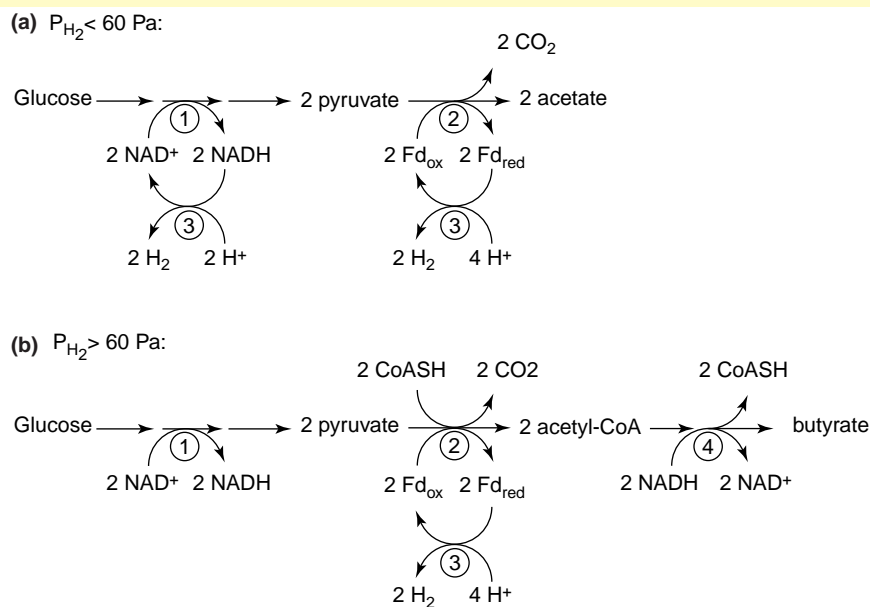
Figure II. Effect of hydrogen partial pressure on biological production of hydrogen. **(a)** Oxidation of NADH by production of hydrogen is thermodynamically favorable only when the hydrogen partial pressure is less than 60 Pa, otherwise; **(b)** other fermentation products must be formed. Reactions [(a) and (b)]: 1, glucose metabolism through glycolysis or the Entner-Doudoroff pathway; 2, oxidative decarboxylation of pyruvate by pyruvate:ferredoxin oxidoreductase; 3, formation of hydrogen by hydrogenase; 4, butyrate fermentation.

equivalents (electrons) to an electrode rather than to an electron-acceptor molecule.

Several mechanisms have been described by which electrons can be transferred to metals or electrodes. For example, the dissimilatory metal-reducing bacterium (DMRB) *Geobacter* spp. makes direct physical contact with solid electron acceptors and uses periplasmic c-type cytochrome proteins as the metal reductase [28,29]; hence, these organisms must grow as a biofilm on the electrode surface [30]. Other types of DMRB, such as

Shewanella spp., can transfer electrons to solid acceptors through either direct contact or by production of soluble quinones that act as electron-shuttling compounds [31,32]. For organisms of this type, direct contact with the electrode surface is not required.

In addition to microorganisms that can transfer electrons to the anode, the presence of other organisms appears to benefit MFC performance. One research group found that a mixed culture generated a current that was sixfold higher than that generated by a pure culture [33].

Table 2. Maximum hydrogen yields achieved from organic material by a mixed culture performing dark fermentation during optimization efforts

Optimization effort	Reactor type	Substrate	Max. Hydrogen yield (mol/mol) based on hexose	References
Initial pH and acetic/butyric acid	Batch	Sucrose/starch	1.8	[61]
Reactor configuration	Fluidized bed reactor	Sucrose	1.3	[62]
Hydrogen partial pressure	CSTR	Wheat starch	1.9	[20]
Inhibition of acetic/butyric acid	Batch-fed	Glucose	2.0	[63]
Reactor operation, temperature	Upflow reactor	Wastewater	2.1	[13]
Immobilized biomass	Batch	Sucrose	2.0	[64]
Immobilized biomass, granules	Fermentor	Sucrose	2.1	[16]
pH	Fermentor	Glucose	2.1	[65]
Hydrogen partial pressure, substrate	Batch	Sucrose, lactate	0.5	[11]
Hydraulic retention time	CSTR	Glucose, sucrose	2.2	[66]
Peptone addition	Batch/chemostat	Cellulose	2.0	[15]
Nitrogen source	Batch	Glucose	2.4	[67]
pH and substrate levels	Batch	Sucrose	2.5	[21]
Hydrogen partial pressure	CSTR	Glucose	1.4	[68]
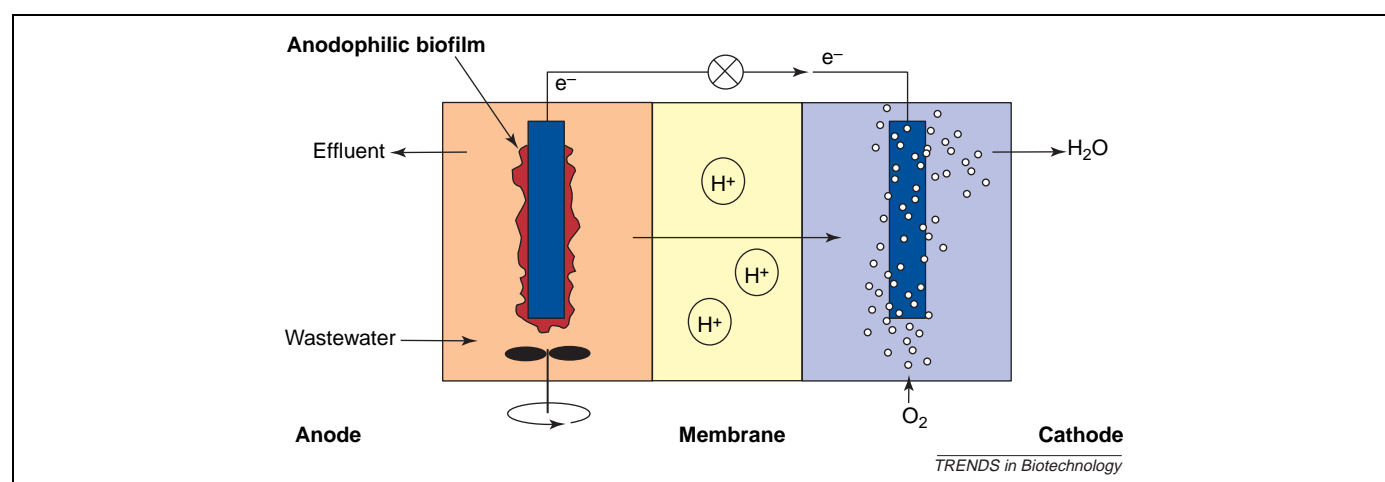
Figure 1. Schematic of a dual-chamber microbial fuel cell (MFC). Anaerobic microorganisms oxidize organic material in the anode chamber. Some microorganisms attach to the electrode as a biofilm and the electrons are transferred directly to the electrode and then through a wire to the cathode where the electrons reduce oxygen to water. Protons are transferred in the same direction through a proton-exchange membrane. Mixing is required in the anode chamber, whereas aeration is required in the cathode chamber.

Hence, the microbial communities that develop in the anode chamber may have a similar function as those found in methanogenic anaerobic digesters, except that microorganisms that can transfer electrons to the electrode surface replace methanogens. Rabaey and co-workers [23] referred to such microbial communities as adapted anodophilic consortia. Anodophilic bacteria from different evolutionary lineages from the families of *Geobacteraceae*, *Desulfuromonaceae*, *Alteromonadaceae*, *Enterobacteriaceae*, *Pasteurellaceae*, *Clostridiaceae*, *Aeromonadaceae*, and *Comamonadaceae* were able to transfer electrons to electrodes [24,26,30,33–38].

The major limitations to implementation of MFCs for treatment of wastewater are that their power density is still relatively low and the technology is only in the laboratory phase. Based on the potential difference, ΔE, between the electron donor and acceptor, a maximum potential of ~ 1 V can be expected in MFCs, which is not much greater than the 0.7 V that is currently being produced [39]. However, by linking several MFCs

Table 3. Maximum power densities achieved in MFCs using various substrates during optimization efforts

Optimization effort	Chamber	Inoculum	Substrate	Max. power density[a] (mW/m^2)	References
Cathode performance	Single	Wastewater	Wastewater	26	[40]
Anodophile consortium selection	Dual	Anaerobic sludge	Glucose	3,600	[23]
MFC configuration, electrodes	Single	Anaerobic sludge	Glucose	788	[39]
Specific inoculum	Dual	*Geobacter* sp.	Acetic acid	15	[30]
Electrode surface area, substrate	Dual	*Rhodoferax* sp.	Glucose	8.2	[26]
Electrode material	Single	Anaerobic sludge	Lactate	10.2	[33]
Concentration of bacteria	Dual	*Shewanella* sp.	Lactate	3.6	[37]
Electron shuttles, physiological state	Dual	*Escherichia coli*	Glucose	0.5	[36]

[a]Expressed as power density, which is power per unit area of anode electrode surface.

together, the voltage can be increased. Currents and power densities, however, are lower than what is theoretically possible, and system performance varies considerably (Table 3). The maximum power density reported in the literature (3600 mW/m^2) was observed in a dual-chamber fuel-cell treating glucose with an adapted anaerobic consortium in the anode chamber and a continuously aerated cathode chamber containing an electrolyte solution that was formulated to improve oxygen transfer to the cathode. Costs were minimized by using plain graphite electrodes without exogenous electron shuttles and a commercially available exchange membrane [Ultrex™ ion-exchange membrane (Membranes International, Inc.; http://www.membranesinternational. com/)] [23]. Further improvement in power density is required, and the rates of electron transport to the anode electrode are thought to be a major limiting factor [23]. Optimization of MFCs involves investigation of a variety of aspects of their operation, as given in Table 3.

For wastewater treatment in MFCs to be feasible, the construction and operating costs must be reduced [23,40]. The requirement for expensive noble metals in electrodes, and soluble or electrode-bound electron shuttles are two important cost elements that are being addressed by current researchers [23,39,40]. Single-chamber MFCs with air as the cathode chamber circumvents the cost of aeration [39,40]. In addition, the rate of electron transport must be improved; this may be achieved by selecting a well-adapted anodophilic microbial community and optimizing the MFC operating conditions. Optimization can be conducted in a systematic fashion only when the mechanisms of electron transfer from microorganism to electrode are better understood. Even when optimization is achieved, it remains to be seen whether reactor size can be small enough to make direct bioelectricity production by MFCs economically viable.

Biological chemical production

A major limitation to wider application of the bioenergy technologies described in the previous sections is the relative low cost of the current non-renewable energy sources. Government subsidies, or a direct local need to save on energy costs (for example, biogas that is used directly on-site as a fuel), are necessary to make those processes economically viable. In addition, although bioenergy production may reduce the cost of wastewater treatment, it cannot entirely satisfy the energy demand of our society. Therefore, the production of high-value chemicals from organic material in wastewater might be more feasible than bioenergy production [41]. Industrial wastes can become inexpensive raw materials for integrated fermentation processes [42–44]. High-carbohydrate wastewaters that are unsuitable for animal or human feeding are particularly appropriate for conversion to valuable products in pure-culture or co-culture processes (e.g. Table 1, reactions 17–19). It is the cost efficiency of the bioconversion process that ultimately determines whether a specific waste stream is suitable for production of a specific product [42]. Examples of biochemicals that are obtained via fermentation of organic material in waste streams are shown in Table 4.

Strategies to enhance bioconversion of organic material from waste streams are the main focus of the studies reported in the literature. The poor conversion yield is perhaps the greatest barrier, and therefore much effort has been devoted to enhancing the amount of product formed per reactor volume, per time period. The main approaches to achieving this goal have been discovery of novel microorganisms, metabolic engineering for heterologous gene expression, protein engineering by mutagenesis and molecular evolution, manipulation of cell genomes, and control of carbon fluxes by the addition of external substrate and inhibitors [45–48].

Bioconversion may also be enhanced by process modification, such as culture immobilization [49,50] or coupling two separate bioreactors. For example, anaerobic (hydrogen) fermentation and aerobic conversion of volatile fatty acids by *Ralstonia eutropha* were combined to enhance the efficiency of polyhydroxyalkanoate production from food wastes [51]. Another promising strategy for increasing waste bioconversion rates is the use of co-cultures of pure microbial strains in a single process. Within this strategy, one species performs most of the complex nutrient hydrolysis, and, in turn, provides its metabolic byproducts to the second species, which forms the desired product [52]. This study achieved high acetic acid yields during bioconversion of milk permeate by combining cells of *Clostridium thermolacticum*, *Moorella thermoautotrophica,* and *Methanothermobacter thermoautotrophicus* in a consortium. Addition of the hydrogenotrophic methanogen decreased the hydrogen partial pressure, which increased the acetic acid production. Another potential advantage of co-cultures is that they might be less sensitive than single cultures to changes in composition of industrial and agricultural wastewaters (i.e. the wastewater may contain multiple organic compounds, the relative concentrations of which may change on a diurnal cycle). Stability in biological waste treatment systems has been linked to increased biodiversity of the microbial community [53] and to an increased ability of the community to use alternative removal pathways for the same substrate [54].

In bioconversion of agricultural and food wastes to valuable chemicals, separation and purification of the products from the bulk liquid represents the highest percentage of the manufacturing cost. Therefore, the economic feasibility of reusing wastes will strongly depend on the downstream processing efficiency. In recent years, considerable advances have been achieved in biomolecule purification technologies; one of the key goals is to achieve more selective, more efficient, and shorter separation routes [55]. For example, a single-step direct lactic acid separation from fermentation broths has been successfully implemented using anionic fluidized-bed columns [56]. Supercritical fluid technology (i.e. the use of a fluid at a temperature and pressure that are greater than its critical levels) has also proven to be a useful tool for achieving separation of compounds directly from cultures, and is an attractive option because separation is usually performed at ambient temperatures using non-toxic, non-flammable solvents, while achieving product crystallization [57].

Table 4. Biochemical production from agricultural and food industry waste streams by different microbial strains

Waste stream	Industry	Biochemical	Function	Microbial strain(s)[a]	References
Corn steep liquor	Corn wet milling	Lipase	Enzyme	*Galactomyces geotrichum*[b]	[69]
Whey	Wheat milling	Protease	Enzyme	*Mucor* spp.[b]	[70]
Gluten free effluent	Wheat milling	Glycerol	Solvent	*Pichia farinosa*[b]	[43]
Sugar cane bagasse	Sugar	Xylitol	Sweetener	*Pichia guilliermondii*[b]	[49]
Starch-processing	Starch	Biomass protein	Feed	6 microfungi (*Rhizopus* sp.)	[71]
Salad oil	Vegetable oil	Biomass protein	Feed	5 yeasts	[72]
Gluten free effluent	Wheat milling	*Monascus* pigment	Dye	*Monascus purpureus*[b]	[73]
Milk permeate	Dairy	Acetic acid	Deicing salt	*Clostridium stercorarium* subsp. *Thermolacticum*[c] plus *Moorella thermoautotrophica*[c] plus *Methanothermobacter thermoautotrophicus*[d,e]	[52]
Gluten free effluent	Wheat milling	Lactic acid	Bioplastic	*Lactobacillus bulgaricus*[c]	[43]
Food	Agricultural	Polyhydroxyalkanoates	Bioplastic	*Ralstonia eutropha*[c]	[51]
Whey	Dairy	Polyhydroxyalkanoates	Bioplastic	Recombinant *E. coli*[c]	[47]
Mussel-processing	Fish	Nisin, Pediocin	Antibacterials	*Lactococcus lactis*[c], *Pediococcus acidilactici*[c]	[74]

[a]According to current nomenclature
[b]Fungal strain
[c]Bacterium
[d]Archaeaon
[e]In a co-culture

Table 5. Comparison of the four bioprocessing strategies for wastewater treatment

Bioprocess strategy	Level of maturity	Separation of products	Culture	Value added
Anaerobic digestion	Mature, operational	Easy, gas	Mixed	Low
Hydrogen fermentation	Laboratory phase	Easy, gas	Mixed	Low to medium
MFC	Laboratory phase	Easy, electricity	Mixed	Low
Biochemical production	Scale-up phase	Hard, soluble products	Pure or co	Medium to high

Outlook

Anaerobic digestion of industrial and agricultural wastewater to methane is a mature process that is being used within full-scale facilities worldwide. Although methane is a low-value product (Table 5), methanogenic anaerobic digestion may still represent the most economically viable technology, because catalytic conversion of biogas (i.e. methane) to syngas (i.e. hydrogen and carbon monoxide) is relatively simple. Syngas can be used to produce liquid fuel and high-value products through conventional chemical manufacturing processes. Hydrogen production via dark fermentation has the greatest potential as a pre-treatment step that can be followed by a suitable secondary process step, such as bioconversion of volatile fatty acids to other products, such as polyhydroxyalkanoates. The possibility of direct conversion of organic material in wastewater to bioelectricity is exciting, but fundamental understanding of the microbiology and further development of the technology is required.

Methanogenic anaerobic digestion, hydrogen fermentation and bioelectricity production all use a mixed microbial community that is selected according to function. This is well-suited to the non-sterile, ever-changing, complex environment of wastewater treatment. Also, the products from these bioprocesses can be easily separated as gases or bioelectricity (Table 5). However, in our current, cheap-energy economy, such large-scale implementation may not be economically feasible without further conversion to more valuable products. Fortunately, specialized high-value biochemicals might soon be produced from wastewater to elevate bioprocessing as an economically viable choice. Issues concerning the separation of the soluble products from the fermentation broth, and the stability of pure- or co-culture fermentation processes remain to be addressed. Furthermore, the effect of bioreactor design configuration and operating conditions (e.g. biomass retention, mixing) need to be more widely studied and optimized before the processes can be scaled up. The biological oxygen demand in the effluent, which is an indication of how well wastewater is treated, will be too high in all four different bioprocessing strategies, and thus, post-treatment with, for example, activated sludge, is an anticipated requirement. Hence, post-treatment facilities must to be integrated into the design of full-scale bioprocessing operations.
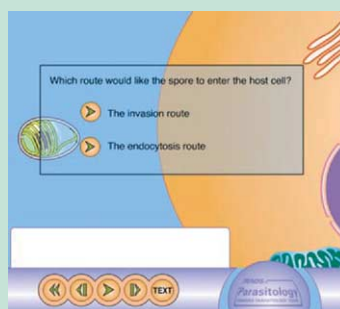
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74 Guerra, N.P. and Pastrana, L. (2002) Nisin and pediocin pro-duction on mussel-processing waste supplemented with glucose and five 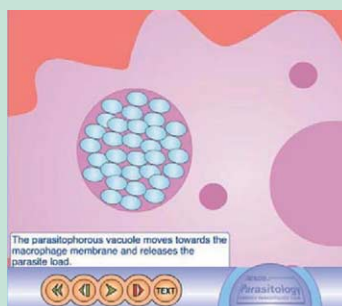nitrogen sources. *Lett. Appl. Microbiol.* 34, 114–118