Green Energy and Technology

Kasthurirangan Gopalakrishnan
J. (Hans) van Leeuwen
Robert C. Brown *Editors*

Sustainable Bioenergy and Bioproducts

Value Added Engineering Applications

Springer

Green Energy and Technology

For further volumes:
http://www.springer.com/series/8059

Kasthurirangan Gopalakrishnan
J. (Hans) van Leeuwen · Robert C. Brown
Editors

Sustainable Bioenergy and Bioproducts

Value Added Engineering Applications

Springer

Dr. Kasthurirangan Gopalakrishnan
Department of Civil and Construction Engineering
Iowa State University
Ames IA
USA
e-mail: rangan@iastate.edu

J. (Hans) van Leeuwen
Iowa State University
Town Engineering Building 376
Ames IA 50011
USA
e-mail: leeuwen@iastate.edu

Robert C. Brown
Iowa State University
Marston Hall 411
Ames IA 50011
USA
e-mail: rcbrown@iastate.edu

ISSN 1865-3529
ISBN 978-1-4471-2323-1
DOI 10.1007/978-1-4471-2324-8
Springer London Dordrecht Heidelberg New York

e-ISSN 1865-3537
e-ISBN 978-1-4471-2324-8

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Control Number: 2011941718

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

The twenty-first century saw rapid expansion into making liquid fuel production more sustainable by producing ethanol from corn starch. Brazil already pioneered ethanol from sugar cane, but a logical step was to use corn from the world's most prolific agricultural production area, the USA's Midwest. Ethanol production in the US jumped in a few years from a few billion gallons to fourteen billion gallons, all in less than a decade, to become the world leaders in biofuels production. This is a remarkable achievement of free enterprise, mainly driven by smaller companies and farmer owned consortia. While corn is not the answer to replacing fossil fuel sources due to production limitations, the industry made a brave statement in replacing almost 10% of all liquid fuel needs. What is more, ethanol is a very clean octane enhancer and avoids the pollution of water resources found with the previously and still used MTBE (methyl tertiary butyl ether) from petroleum sources. The production cost of ethanol from corn is not quite competitive with petroleum-based gasoline and the industry has been relying heavily on selling coproducts, particularly distillers dried grains (DDG) and subsidies. With subsidies being withdrawn, the industry will become even more dependent on efficient coproduct utilization.

Biofuels have come under scrutiny and criticism recently. It is claimed that although renewable, these products are not as environmentally friendly as originally conceived, mainly because the energy inputs, often from non-renewable sources, are as large or larger than the energy contained in the fuel product. In addition, corn used in ethanol production reduces the supply of a staple food or animal feed. Lastly, there is a perception that the US ethanol causes an increase in food prices. Careful analysis of all these allegations will show that these are generally exaggerated and the ethanol industry deserves much more praise for their pioneering efforts in ethanol production. What is more, the industry deserves to be supported by research into better utilization of all the organic matter to produce additional coproducts some of which is presented in this book. Nevertheless, there is a clear need to expand biofuel production beyond what can be based on seeds.

Lignocellulosic ethanol production seems to have the key to sustainable biofuel production. Taken as a whole, with billions of tons of primary plant productivity of

lignocellulosic material per year world-wide, it becomes clear that such quantities of raw material could indeed become the main raw material for liquid fuel production. The production capacity of hydrocarbons by the plant kingdom, algae and cyanobacteria using solar energy is huge and the first chapter describes mechanisms of such primary production. However, while the technology exists, current production methods, on the verge of intermediate-scale production, are not yet economical and would indeed be more costly than ethanol production from corn. Various methodologies are being developed to lower costs and some chapters in this book are devoted to this topic, including electrochemical pathways to produce electricity based on simple degradation products from lignocellulosics.

Equally important is the fact that all biofuel production methods generate leftovers. These leftovers do not only present disposal challenges, but also provide opportunities for making further coproducts. These coproducts could play even further important roles in substituting for petroleum products in making biopolymers and many other valuable substances. Only when all organic material from lignocellulosic material is used gainfully will the biofuel industry truly become sustainable. Various chapters in this book are devoted to further useful applications of the leftovers from various lignocellulosic processing operations under development.

The last three chapters reflect on the overall picture of use and production of biofuels and its infrastructure impacts, assessment of environmental risks and the lifecycle of biofuels.

Researchers and practitioners engaged in the study of bioenergy and bioproducts will find this book very useful. This book will also serve as an excellent state-of-the-art reference material for graduate and postgraduate students in biorenewables.

Contents

About the Editors

Kasthurirangan Gopalakrishnan Ph.D.
Prof. Kasthurirangan Gopalakrishnan is a Research Assistant Professor in the Department of Civil, Construction and Environmental Engineering at Iowa State University. He received his Ph.D. in Civil Engineering from the University of Illinois at Urbana-Champaign in 2004. His research interests include sustainable infrastructure, green engineering technology, bio-inspired computing and smart pavements. Dr. Gopalakrishnan has published the textbook, *Sustainable Highways, Pavements and Materials*: *An Introduction*, and is also the lead editor of Springer's *Sustainable and Resilient Critical Infrastructure Systems*: *Simulation*, *Modeling*, *and Intelligent Engineering*, and *Soft Computing in Green and Renewable Energy Systems*. He is also an Associate Editor of *International Journal for Traffic and Transport Engineering*.

J. (Hans) van Leeuwen Ph.D.
Dr. J. (Hans) van Leeuwen is Vlasta Klima Balloun Professor of Environmental and Bioengineering at Iowa State University. He received his Ph.D. in from University of Pretoria in 1988. He is the 2009 *R&D Magazine Innovator of the Year* and he received the American Academy of Environmental Engineers' *Grand Prize for University Research* in 2007, 2008 and 2009. Dr. van Leeuwen is the co-inventor and owner of the MycoMeal fungal process and the MellO3z alcohol purification process and holds several patents in his area of research which mainly focuses on biorenewables, value-adding processing and ozonation applications. Dr. van Leeuwen has also received the *R&D 100 Award* in 2008 and 2009 and *Iowa State University Award for Outstanding Achievement in Research* in 2009. He is an Editorial Board Member of the *International Journal of Environment and Waste Management* and *Ozone Science and Engineering* as well as a Fellow of four international professional societies.

Robert C. Brown Ph.D.
Dr. Robert Brown is Anson Marston Distinguished Professor of Engineering and Gary and Donna Hoover Chair in Mechanical Engineering at Iowa State

University. He is the director of ISU's Bioeconomy Institute which coordinates research, education and extension in bioenergy and bio-based products at ISU. His research focuses on the thermochemical processing of biomass and fossil fuels into energy, fuels and chemicals. He has published the textbook *Biorenewable Resources*: *Engineering New Products from Agriculture* and helped establish at ISU the first graduate program in the United States to offer degrees in biorenewable resources. Dr. Brown is a Fellow of the American Society of Mechanical Engineering (ASME) and Distinguished Iowa Scientist of the Iowa Academy of Science. He is also the recipient of the *David R. Boylan Eminent Faculty Award for Research* at ISU in 2002.

Linear Hydrocarbon Producing Pathways in Plants, Algae and Microbes

Mark Brown and Jacqueline Shanks

Abstract Many different types of organisms synthesize hydrocarbons in nature, but for all their ubiquity, the biochemical and genetic bases for how these compounds are synthesized are not well understood. Several biochemical mechanisms have been proposed for non-isoprenoid hydrocarbon biosynthesis, most notably the head-to-head condensation and elongation-decarboxylation pathways from fatty acid precursors, but definitive characterization of these and other possible mechanisms have largely remained elusive. This review explores the possible metabolic pathways that various plant, algal, and microbial species use to synthesize linear hydrocarbons and the genetic factors that are involved in regulating those pathways.

1 Introduction

It has long been known that hydrocarbons from plants, algal, and microbial organisms are present in crude oil deposits [7, 23]. This was originally recognized for the possibility of using these molecules as evolutionary indicators, since evolutionary processes can be deduced from the biochemistry of distinct compounds [11]. While the substantial portion of these deposits is the result of the

M. Brown
Department of Chemical and Biological Engineering 4130 Biorenewables Research Laboratory, Iowa State University, Ames IA 50011, USA
e-mail: brownmt@iastate.edu

J. Shanks (✉)
Department of Chemical and Biological Engineering, 4135 Biorenewables Research Laboratory, Iowa State University, Ames IA 50011, USA
e-mail: jshanks@iastate.edu

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*, Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_1,

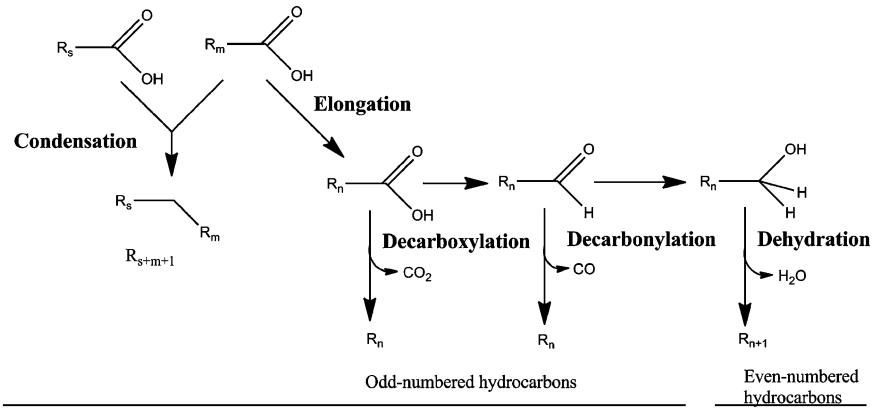

Fig. 1 Possible pathways for long-chain hydrocarbon biosynthesis from a fatty acid precursor

breakdown of biomass over time, these original hydrocarbons maintain their integrity due to their extremely stable nature [6]. In some cases, these hydrocarbon markers are still viable in deposits that are over three billion years old [3]. Recently though, interest in the biosynthesis of hydrocarbons has grown substantially due to the prospect of using hydrocarbon producing organisms as a source of renewable fuels.

Long-chain hydrocarbons, consisting of alkanes and alkenes, are promising targets for biofuels as they are already the major constituents of petroleum-based fuels that are in use today. Synthesis of these compounds in nature is accomplished by an assortment of different types of organisms and play a variety of roles, as insect epicuticular waxes and pheromones [15, 30], protective coatings on fungal spores [35], epicuticular waxes covering stems and leaves in higher plants [18, 26], and various other purposes in many microorganisms, including blue-green and green algae, cyanobacteria, and yeasts, among others [11, 16].

2 Hydrocarbon Biosynthesis

It has been well documented that fatty acid metabolites are precursors to long-chain hydrocarbons. This has been demonstrated through radiocarbon labeling studies in a multitude of species [17, 33]. While many details are not well characterized, there appears to be two main metabolic pathways for the synthesis of hydrocarbons from fatty acids (Fig. 1).

The first, primarily found in bacteria, is head-to-head condensation of two fatty acids [2, 4, 22]. In this putative pathway, a bond is formed between the carboxyl carbon of one fatty acid and the α-carbon of a second fatty acid. The resulting compound is then decarboxylated to form a hydrocarbon that is one carbon less

than the total carbons from the two fatty acids [1]. The ensuing hydrocarbon chain length would be odd assuming the number of carbons in the fatty acid groups was even, as is typically the case.

The second pathway, evidence of which has been found in many plant and algal species [10, 20, 25], is the elongation-decarboxylation mechanism. It is characterized by the elongation of the precursor fatty acid (primarily C_{16} or C_{18}) to a very long-chain fatty acid (VLCFA, longer than C_{22}) in a process analogous to *de novo* fatty acid synthesis. There are several possible mechanisms from which the hydrocarbon could then be formed from the VLCFA. The first is decarboxylation, in which the carboxylic end of the VLCFA is cleaved to form a hydrocarbon that is one carbon shorter than the VLCFA. A second possibility is reduction of the carboxylic end of the VLCFA to an aldehyde, with subsequent decarbonylation to a hydrocarbon identical to that formed from decarboxylation. The final mechanism is reduction of the aldehyde to a primary alcohol, which could then be dehydrated to the resulting hydrocarbon without the loss of the terminal carbon. These pathways are discussed in more detail in the ensuing sections.

2.1 Head-to-Head Condensation

Early investigations of the head-to-head condensation mechanism of hydrocarbon biosynthesis took place in the late 1960s. Incorporation of ^{14}C-labeled fatty acids in the tobacco plant [17] and bacterium *Sarcina lutea* [1] suggested that two fatty acids were being incorporated into the final hydrocarbon. Additionally, cell extracts have shown that the carboxyl group for only one of the fatty acids was being lost as carbon dioxide while the other was retained in the hydrocarbon. Together, these results were consistent with head-to-head condensation over alternative mechanisms such as elongation-decarboxylation, but the underlying biochemistry and genetics were not revealed with any clarity. Only recently have further studies shed light on the underlying basis for this chemistry.

One such study looked at hydrocarbon synthesis in the bacterium *Micrococcus luteus*, a close relative to the previously studied *S. lutea*, for which a genome sequence was available. A search for homologs of the KAS enzymes responsible for condensation of fatty acyl groups in de novo fatty acid synthesis resulted in a single target gene (Mlut_13230) that showed promise in heterologous expression in *E. coli*. Due to its sequence similarity to KAS III, the enzyme responsible for catalyzing the initial condensation reaction in fatty acid synthesis, a mechanism for head-to-head condensation was proposed that is very similar to the fatty acid synthesis reaction (Fig. 2). One fatty acid is initially oxidized to a β-ketoacyl-CoA (similar to malonyl-CoA), followed by a decarboxylative Claisen condensation with the second fatty acid in the form of fatty acyl-CoA, catalyzed by Mlut_13230. The condensed product is theorized to be a diketone, with three subsequent reductions and two dehydrations needed to form the monounsaturated hydrocarbon, reactions similar to the reduction and dehydration reactions that take place

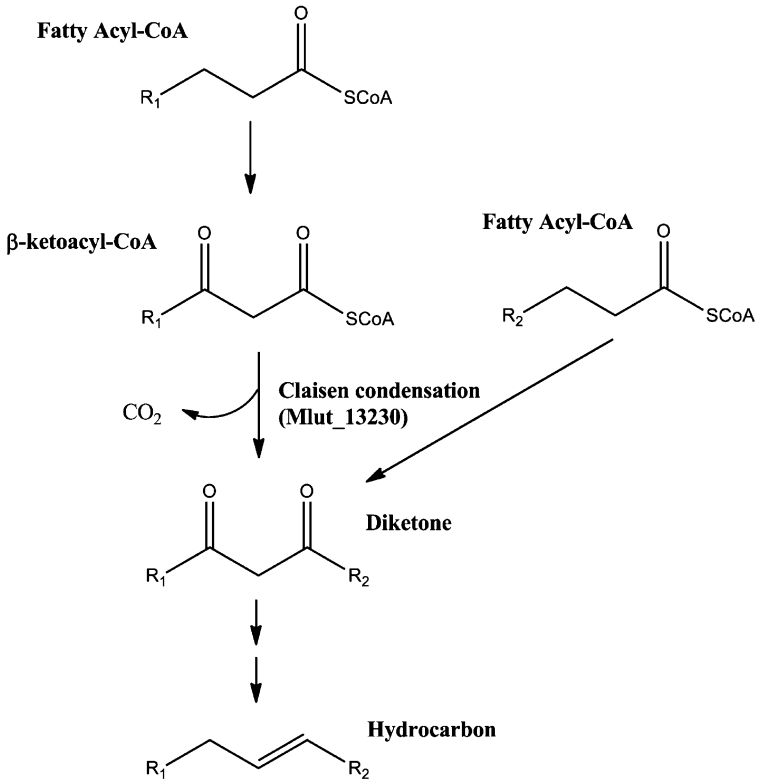

Fig. 2 Possible head-to-head condensation mechanism, where R1 and R2 are alkyl groups of the precursor fatty acid. The monoketones observed in vitro were hypothesized to occur as an intermediate in the reduction and dehydration steps between the diketone and the hydrocarbon [4]

in fatty acid synthesis. In vitro studies with the purified Mlut_13230 protein, tetradecanoyl-CoA, and *E. coli* lysate resulted in unsaturated C_{27} monoketones, a possible intermediate between the diketone and the final hydrocarbon in Fig. 2 [4]. Enzymes catalyzing the initial oxidation steps, as well as the reduction and dehydration steps at the end of the possible pathway were not found, however, leaving many of the proposed mechanisms unsupported. Furthermore, two additional *M. luteus* genes (Mlut_13240 and Mlut_13250) were found to be required for successful heterologous expression in *E. coli,* yet no clear function for them was found.

Soon after the preceding pathway was put forth, a separate investigation came out with substantially different conclusions as to the underlying mechanisms for head-to-head condensation. In that study, an *oleABCD* gene family was identified as necessary for head-to-head hydrocarbon biosynthesis. Of the 3,558 genomes surveyed, only 69 were qualified as potential candidates for having hydrocarbon producing *ole* genes. All the positive results were from bacterial genomes, while

none of the more than 2,100 *Eukaryota* and 84 *Archaea* genomes showed strong evidence for containing the *oleABCD* gene family [31]. Of the four *ole* genes identified as being integral to head-to-head condensation, *oleA* was the primary candidate for the catalysis of the condensing reaction, as it was homologous to the thiolase superfamily of condensing enzymes. Additionally, the *Shewanella oneidensis oleA* gene was found to have 31% sequence similarity to the corresponding Mlut_13230 condensing enzyme in *M. luteus* [32]. Up to six different gene arrangements were found for *oleABCD*, the highest population consisted of an unbroken cluster of all four genes arranged continuously, while second was the fusion of the *oleBC* genes into one gene within the larger cluster (a configuration that *M. luteus* was shown to exhibit, hence the three genes found as in the previous study). Various other cluster arrangements and dispersion of individual genes throughout the genome were observed as well, but with much less species exhibiting those characteristics. Despite the large difference in populations, each of the six arrangements of genes had at least one bacterial species that produced hydrocarbons. While different compounds were created, all were greater in length than C_{23} and had at least one unsaturation, consistent with head-to-head synthesis. Among the 14 different types of bacteria tested, 10 had a single product with a carbon length of 31 with nine double bonds known as 3, 6, 9, 12, 15, 19, 22, 25, 28-hentriaconanonaene. This is consistent with the condensation of two molecules of the same hexadecatetraenoic acid, a compound known to be an intermediate in the biosynthesis of long-chain polyunsaturated fatty acids (PUFAs) that are produced in various *Shewanella* strains that were a part of a parallel study [32]. This link was confirmed when the PUFA synthesis pathway was blocked in a mutant strain and no hydrocarbons could be detected, while restoration of the blocked gene restored hydrocarbon biosynthesis.

The fact that many of the bacteria produced just a single hydrocarbon compound from a relatively non-abundant fatty acid precursor points to a probable substrate specificity for the *oleA* protein. A series of heterologous expression experiments of *Stenotrophomona maltophilias oleA* in *Shewanella oneidensis* showed that not only was *oleA* selective, but the entire gene cluster was as well. Additionally, the *S. maltophilia oleA* showed specificity for saturated and monounsaturated fatty acids by synthesizing mono and di-unsaturated ketones. The corresponding *oleBCD* gene set was not present to metabolize the intermediates, so the ketones were the final product of the heterologous expression. The native *oleA* was only able to produce the C_{31} ketone with eight double bonds when a mutant *S. oneidensis* was created without *oleBCD* genes. When present, the final product was the hentriaconanonaene [31].

While the previously presented mechanism proposed a decarboxylative Claisen condensation due to homology of the *oleA* gene to KAS III, the thiolase superfamily of genes catalyzes both decarboxylative and nondecarboxylative condensation reactions [14]. An alternative mechanism consisting of the nondecarboxylative reaction was therefore offered (Fig. 3). Based on the experimental data, it is believed that *oleA* catalyzes the first reaction where the two fatty acyl groups are condensed to the β-keto thioester intermediate. Absence of the *oleBCD* genes could

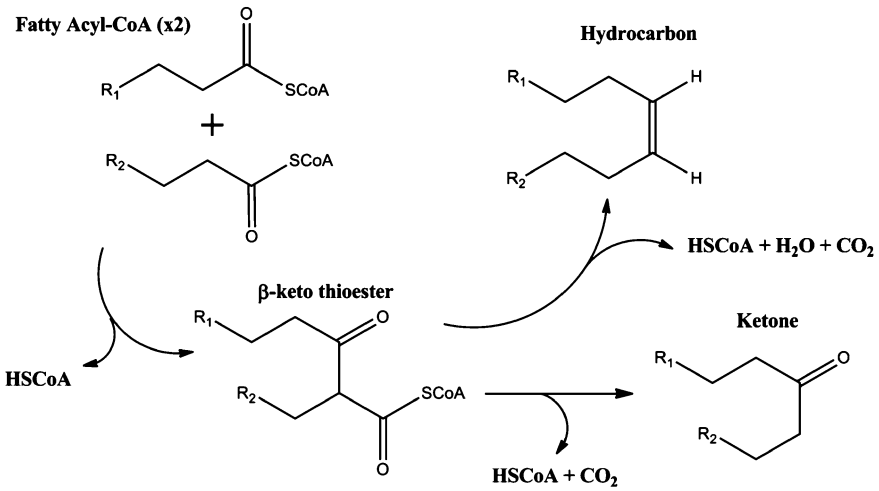

Fig. 3 A second possible fatty acyl head-to-head condensation mechanism. The nondecarboxylative Claisen condensation takes place first through the action of *oleA*, producing the *β*-keto thioester. *OleBCD* presumably catalyze the reactions that lead to the hydrocarbon, while in their absence the *β*-keto thioester could undergo hydrolysis and then spontaneously decarboxylate to the ketone (adapted from Sukovich et al. (22))

allow the *β*-keto intermediate to undergo hydrolysis to an unstable *β*-keto acid, which can spontaneously decarboxylate to the ketones observed in the heterologous expression experiments discussed previously [32]. The presence of the appropriate *oleBCD* genes would preclude this branch mechanism, channeling the *β*-keto thioester intermediate to the hydrocarbon instead. There is some indication of the possible underlying mechanisms based on the known gene families of the *oleBCD* enzymes, but these individual steps have not been described at this point.

2.2 Elongation

Investigation of the so-called "elongation decarboxylation" pathway has basically followed the same course as that of head-to-head condensation. Early studies indicating this mechanism were first accomplished in the 1960s, with incorporation of radiocarbon-labeled fatty acids or acetates used as the main tools for distinguishing between the condensation and elongation mechanisms [2, 18, 19]. This mechanism is characterized by elongation of precursor fatty acids via the fatty acid elongation pathway to a very long chain fatty acid (VLCFA). An initial fatty acid, such as palmitate (16:0) or oleate (18:1) is elongated in the same manner as *de novo* fatty acid synthesis, with a couple of exceptions [27]. First, malonyl-CoA units are not converted to malonyl-ACP prior to condensation with the acyl-CoA. Secondly, instead of being located in the cytoplasm, as in eukaryotes and bacteria

or in the plastids for photosynthetic organisms for de novo fatty acid synthase, the VLCFA synthase is located in the membrane of the endoplasmic reticulum (ER). The mechanism for elongation behaves mostly in the same way as de novo synthesis though, with repeated malonyl-CoA units added to the growing acyl-CoA chain until the desired length is reached, typically 24–34 carbons long [28]. In order to make the hydrocarbon from the resulting VLCFA, the carboxylic end must be replaced. There are several possible mechanisms for which this can happen.

2.2.1 Decarboxylation

Until the 1980s, elongation-decarboxylation was the only mechanism considered for converting VLCFAs to hydrocarbons. While it was known that the carboxylic carbon was lost in hydrocarbon synthesis, the direct cleavage of the carboxyl end was not considered feasible without an electron withdrawing group to stabilize the negative charge on the leaving CO_2 [9]. It was thought that such a group would exist in the immediate precursor to the decarboxylic reaction, but this has never been detected [21]. In the 1970s, experimental results started to show that perhaps an aldehyde was a precursor to the hydrocarbon instead [5], but the mechanism by which that would happen was unknown. It was not until the mid 1980s that a possible reduction-decarbonylation mechanism was first suggested [8].

2.2.2 Reduction-Decarbonylation

Inhibition of alkane synthesis in young leaves of the plant *Pisum sativum* was the first indication that aldehydes were an intermediate to hydrocarbon synthesis, though this was not recognized at the time [5]. Addition of metal ion chelators inhibited the normal synthesis of the alkane hentriacontane, with a C32 aldehyde accumulating instead. Since decarboxylation was the consensus mechanism at the time, this was thought to be a side reaction of an intermediate in that pathway. Subsequent experiments in *P. sativum* have shown that the aldehyde is likely the immediate precursor to the hydrocarbon. In cell-free preparations, the microsomal fraction (inclusive of ER remnants) was found to convert octadecanoyl-CoA to the aldehyde octadecanal along with some hydrocarbon synthesis activity. The heavy particulate fraction (inclusive of cell wall, cutin, and other membranes) did not have any activity with the acyl-CoA, but readily converted exogenous aldehyde to alkanes [8]. In concert with hydrocarbon synthesis activity, CO was measured as a byproduct in nearly a 1:1 mol ratio with produced hydrocarbon while no CO_2 was indicated, further demonstrating a decarbonylation mechanism over decarboxylation.

Similar enzymatic activity was observed in the algae *Botryococcus braunii*. A purified aldehyde decarbonylase was shown to produce CO in nearly a 1:1 stoichiometry with the alkane formed. Crude preparations, however, produced mainly CO_2 instead of CO, something not observed in the previous *P. sativum* (pea)

experiments. It was theorized that the algae had evolved a CO oxidizing mechanism to circumvent the toxic effects of CO, while the probable location of the decarbonylase in the cuticle of peas would allow for a direct venting to atmosphere and no need for the oxidizing action [9].

Several other organisms have been studied for the presence of a decarbonylase as well, including the cyanobacteria *Synechococcus elongatus* and plant *Arabidopsis thaliana*. In the latter, a *cer1* wax deficient mutant was found to have an increase in aldehydes and a drop in alkanes from the wild-type [12]. While one would expect this behavior if an aldehyde decarbonylase was deficient in that mutant, the same relationship could be seen if the aldehydes were a side product of the main pathway [28]. Subsequent analysis of the wax production in the cer1 mutant showed that the cer1 deletion could not have been simply a block of the decarbonylation reaction [13]. In the cyanobacteria *S. elongates*, two candidate genes for alkane biosynthesis, PCC7942_orf1593 and orf1594 were identified as the aldehyde decarbonylase and acyl-CoA reductase, respectively [29]. Heterologous expression of the proposed reductase _orf1594 in *E.coli* produced even-chained fatty aldehydes and alcohols, while expression of the two together resulted in odd-chained alkanes and alkenes. As would be expected when no precursor was present, expression of _orf1593 alone was indistinguishable from the negative control. Expression of _orf1593 genes from 15 other species of cyanobacteria with _orf1594 from *S. elongatus* produced the same combination of results [29].

While the ability to make alkanes makes the elongation-decarbonylation mechanism unique, alkenes are also a possible hydrocarbon product from this pathway. However, a potential mechanism for their synthesis is often ignored. Does VLCFA elongation start with an unsaturated fatty acid or is the VLCFA desaturated in the process of being reduced and decarbonylated? A recent study looking at wax composition in maize silks suggests that both possibilities may be true. When the double bond was located in the elongated portion (that which was added in VLCFA synthesis) of the hydrocarbon, it was determined that the precursor fatty acid was elongated first and then desaturated. Alternatively, if the double bond was in the original fatty acid portion of the molecule, that position was consistent with the particular unsaturated fatty acid that had entered VLCFA elongation [25].

2.2.3 Reduction-Dehydration

In 2004, a new reduction pathway was proposed that does not result in the loss of the carboxylic end of a VLCFA [24]. Moreover, the bacterium being studied, *Vibrio furnisii* M1, produced even-chained hydrocarbons, a rarity from the elongation pathway. C_{14}-labeling studies at the 1-position of the precursor hexadecanoic acid showed incorporation of label in hexadecanal, hexadecanol, as well as the hydrocarbon hexadecane in cell extracts. This indicates that a consecutive

reduction from the fatty acid to an aldehyde to a primary alcohol is possible, with dehydration of the alcohol forming the hydrocarbon.

A follow-up study was done in 2007 by a different group that seemed to contradict the earlier findings. Several different strains of *V. furnissii* were tested for hydrocarbon synthesis in both cell extracts and in intact cells. However, none produced hydrocarbons, much less in the unique manner described previously. Additionally, the complete genome from *V. furnissii* was sequenced yet no alkane biosynthetic or degradation genes were identified [34].

3 Conclusion

The mechanisms by which organisms synthesize hydrocarbons have been studied for nearly five decades. Two main synthesis pathways have been identified as the most prominent, with head-to-head condensation of fatty acids exhibited primarily in *prokaryote* and elongation-decarbonylation existing throughout the animal domains, but principally in *eukarya*. Recent genetic approaches have shed light on the mechanisms by which hydrocarbons are produced that traditional techniques could not. However, until the key enzymes involved in these pathways are cloned and fully characterized, the mechanistic details of how fatty acids are converted to hydrocarbons will remain unresolved.

Acknowledgments This work was supported in part by National Science Foundation Grant EFRI-0938157. M. Brown is an Iowa State University Plant Sciences Institute Fellow.

References

1. Albro PW, Dittmer JC (1969) Biochemistry of long-chain nonisoprenoid hydrocarbons.3. metabolic relationship of long-chain fatty acids and hydrocarbons and other aspects of hydrocarbon metabolism in Sarcina lutea. Biochemistry 8:1913
2. Albro PW, Dittmer JC (1969) The biochemistry of long-chain, nonisoprenoid hydrocarbons. I. Characterization of the hydrocarbons of Sarcina lutea and the isolation of possible intermediates of biosynthesis. Biochemistry 8:394–404
3. Barghoorn ES (1971) The oldest fossils. Sci Am 224:30
4. Beller HR, Goh EB, Keasling JD (2009) Genes involved in long-chain alkene biosynthesis in micrococcus luteus. Appl Environ Microbiol 76:1212–1223
5. Buckner JS, Kolattukudy PE (1973) Specific inhibition of alkane synthesis with accumulation of very long-chain compounds by dithioerythritol, dithiothreitol, and mercaptoethanol in Pisum-Sativum. Arch Biochem Biophys 156:34–45
6. Calvin M (1969) Chemical evolution; molecular evolution towards the origin of living systems on the earth and elsewhere. Oxford University Press, New York
7. Cane RF (1969) Coorongite and the genesis of oil shale. Geochim Cosmochim Acta 33:257
8. Cheesbrough TM, Kolattukudy PE (1984) Alkane biosynthesis by decarbonylation of aldehydes catalyzed by a particulate preparation from Pisum-Sativum. In: Proceedings of the

national academy of sciences of the United States of America-biological sciences, vol 81. pp 6613–6617
9. Dennis M, Kolattukudy PE (1992) A Cobalt-Porphyrin enzyme converts a fatty aldehyde to a Hydrocarbon and Co. Proc Natl Acad Sci USA 89:5306–5310
10. Dennis MW, Kolattukudy PE (1991) Alkane biosynthesis by decarbonylation of aldehyde catalyzed by a microsomal preparation from Botryococcus braunii. Arch Biochem Biophys 287:268
11. Han J, Calvin M (1969) Hydrocarbon distribution of algae and bacteria, and microbiological activity in sediments. Proc Natl Acad Sci USA 64:436–443
12. Hannoufa A, Mcnevin J, Lemieux B (1993) Epicuticular Waxes of Eceriferum Mutants of Arabidopsis-Thaliana. Phytochemistry 33:851–855
13. Hansen JD, Pyee J, Xia Y, Wen TJ, Robertson DS, Kolattukudy PE, Nikolau BJ, Schnable PS (1997) The glossy1 locus of maize and an epidermis-specific cDNA from Kleinia odora define a class of receptor-like proteins required for the normal accumulation of cuticular waxes. Plant Physiol 113:1091–1100
14. Heath RJ, Rock CO (2002) The Claisen condensation in biology. Nat Prod Rep 19:581–596
15. Howard R, Howard RW (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. Annu Rev Entomol 50:371
16. Kalacheva GS, Zhila NO, Volova TG, Gladyshev MI (2002) The effect of temperature on the lipid composition of Botryococcus. Mikrobiologiia 71:336–344
17. Kaneda T (1968) Biosynthesis of long-chain hydrocarbons. 2. Studies on biosynthetic pathway in tobacco. Biochemistry 7:1194–1202
18. Kolattukudy PE (1966) Biosynthesis of wax in Brassica oleracea. Relation of fatty acids to wax. Biochemistry 5:2265–2275
19. Kolattukudy PE (1967) Mechanisms of synthesis of waxy esters in Broccoli (Brassica Oleracea). Biochemistry 6:2705–2717
20. Kolattukudy PE (1968) Tests whether a head to head condensation mechanism occurs in the biosynthesis of n-hentriacontane, the paraffin of spinach and pea leaves. Plant Physiol 43:1466
21. Ladygina N, Dedyukhina E, Vainshtein M (2006) A review on microbial synthesis of hydrocarbons. Process Biochem 41:1001–1014
22. Markey SP, Tornabene TG (1971) Characterization of branched monounsaturated hydrocarbons of Sarcina lutea and Sarcina flava. Lipids 6:190–195
23. Meinschein WG (1959) Origin of petroleum. The American association of petroleum geologists bulletin 43:925
24. Park MO (2005) New pathway for long-chain n-alkane synthesis via 1-alcohol in Vibrio furnissii M1. Biochem J 187:1426–1429 Feb 2005
25. Perera MADN, Qin WM, Yandeau-Nelson M, Fan L, Dixon P, Nikolau BJ (2010) Biological origins of normal-chain hydrocarbons: a pathway model based on cuticular wax analyses of maize silks. Plant J 64:618–632
26. Post-Beittenmiller D (1996) Biochemistry and molecular biology of wax production in plants. Annu Rev Plant Physiol Plant Mol Biol 47:405–430
27. Riezman H (2007) The long and short of fatty acid synthesis. Cell 130:587–588
28. Samuels L, Kunst L, Jetter R (2008) Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu Rev Plant Biol 59:683–707
29. Schirmer A, Rude MA, Li X, Popova E, del Cardayre SB (2010) Microbial Biosynthesis of Alkanes. Science 329:559–562
30. Singer T, Singer TL (1998) Roles of hydrocarbons in the recognition systems of insects. Am Zool 38:394
31. Sukovich DJ, Seffernick JL, Richman JE, Gralnick JA, Wackett LP (2010) Widespread head-to-head hydrocarbon biosynthesis in bacteria and role of OleA. Appl Environ Microbiol 76:3850–3862

32. Sukovich DJ, Seffernick JL, Richman JE, Hunt KA, Gralnick JA, Wackett LP (2010) Structure, function, and insights into the biosynthesis of a head-to-head hydrocarbon in Shewanella oneidensis strain MR-1. Appl Environ Microbiol 76:3842–3849
33. Tornabene TG, Oro J (1967) 14c incorporation into fatty acids and aliphatic hydrocarbons of Sarcina lutea. J Bacteriol 94:349–358
34. Wackett LP, Frias JA, Seffernick JL, Sukovich DJ, Cameron SM (2007) Genomic and biochemical studies demonstrating the absence of an alkane-producing phenotype in Vibrio furnissii M1. Appl Environ Microbiol 73:7192–7198
35. Weete J, Weete JD (1972) Aliphatic hydrocarbons of the fungi. Phytochemistry 11:1201

Fungal Treatment of Crop Processing Wastewaters with Value-Added Co-Products

J. (Hans) van Leeuwen, Mary L. Rasmussen, Sindhuja Sankaran, Christopher R. Koza, Daniel T. Erickson, Debjani Mitra and Bo Jin

Abstract Conventional biological wastewater treatment generates large amounts of low-value bacterial biomass. The treatment and disposal of this excess bacterial biomass accounts for about 40–60% of wastewater treatment plant operational costs. A different form of biomass with a higher value could significantly change the economics of wastewater treatment. Fungi could offer this benefit over bacteria in selected wastewater treatment processes. The biomass produced during fungal wastewater treatment has, potentially, a much higher value than that from the bacterial activated sludge process. The fungi can be used as a protein source and to derive valuable biochemicals. Various high-value biochemicals are produced by commercial cultivation of fungi under aseptic conditions using expensive substrates. Food processing wastewater is an attractive alternative as a source of low-cost organic matter and nutrients to produce fungi with concomitant wastewater purification. This chapter summarizes various findings in fungal wastewater treatment, particularly focusing on creating new byproducts. This chapter also provides an overview on performance of fungal treatment systems under various operational conditions. Important factors such as pH, temperature, hydraulic and solids retention time, nonaxenic and axenic operation, bacterial contamination and others that affect the fungal treatment system are discussed. The work described

J. (Hans) van Leeuwen (✉) · M. L. Rasmussen · C. R. Koza · D. T. Erickson · D. Mitra
Department of Civil, Construction and Environmental Engineering,
Department of Agricultural and Biosystems Engineering,
Department of Food Science and Human Nutrition,
Iowa State University, Ames, Iowa 50011, USA
e-mail: leeuwen@iastate.edu

S. Sankaran
Citrus Research and Education Center, University of Florida, Lake Alfred, FL, USA

B. Jin
School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, Australia

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*,
Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_2,

culminates in the design and operational experience in operating a pilot plant for beneficiating leftovers from ethanol production from corn. Lastly, production of other valuable biochemicals from fungi as further byproducts is discussed.

1 Introduction

Microbial biodegradation of organic compounds in wastewater is a key process in both natural and engineered systems. Bacteria are the mainstay for organic pollutant removal in a typical engineered biological wastewater treatment system. Conventional aerobic treatment (e.g., the activated sludge process) mineralizes organic compounds into carbon dioxide and water and generates a huge amount of bacterial biomass that is the predominant component of so-called secondary sludge. On average, the activated sludge process produces about 0.4 g biomass per gram chemical oxygen demand (COD) removed [1]. Thus, nearly half of the COD removed from wastewater is actually transformed into new bacterial cells, appreciating that COD is a much higher value than the actual organic substrate concentration. The excess bacterial cells, which have to be removed continuously, have little value and are the largest burden in wastewater treatment. Sludge processing, treatment, and disposal constitute one of the major environmental problems in many countries [2]. In fact, the costs associated with treatment and disposal of excess sludge account for up to 60% of the total wastewater treatment plant operating costs [3].

Anaerobic treatment, by way of digestion, is most often used for reducing excess sludge volumes. This is energy efficient with lower biomass production and partially converts wastewater organics into methane, which can be used as a gaseous fuel. However, this processing does not produce high-value byproducts and is capital intensive [1].

Wastewater treatment places a considerable burden on crop-processing industries, resulting in no other benefits than environmental protection.

Filamentous fungi offer a different opportunity. Various filamentous fungi are often cultivated in food industries as a source of valuable products such as protein and a variety of biochemicals, using relatively expensive substrates such as starch or molasses [4]. The use of filamentous fungi to treat high-strength wastewater as a substrate is an attractive option. Fungal cultivation can convert the wastewater organic substances into readily harvestable fungal biomass, which can be used as a source of animal feed and potentially in human diets [5].

Fungi can produce a wide range of fine biochemicals and enzymes, and are more effective than bacteria in metabolizing complex carbohydrates such as starch [6–8]. The possibility of wastewater purification using yeasts and molds for microbial biomass protein (MBP) production has been investigated for some time [6, 9, 10]. The concept of using yeasts in the bioconversion of wastewater has attracted the attention of many researchers due to their ease of cultivation, ability to grow at pH values lower than 5, and growth rates faster than those of molds [11, 12]. In addition, yeasts are less susceptible to contamination by other

microorganisms and producing biomass with high nutritive value [13]. Molds have been considered less suitable than yeasts for MBP production. However, the filamentous nature of these fungi simplifies separation and recovery of the MBP from culture media. Moreover, the obligatory acidophilic properties of these organisms suggest that the fungi would not act as opportunistic pathogens [7, 14]. Mycotoxins produced by pathogenic fungi through secondary metabolic processes, usually to eliminate other microorganisms competing in the same environment, could be a concern [15]. However, such fungi can be eliminated as candidates for cultivation. Thus, filamentous fungi could have interesting benefits for industrial wastewater treatment processes.

Fungi have other advantages over bacteria in biological wastewater treatment, in addition to being a source of valuable fungal byproducts such as amylase, chitin, chitosan, glucosamine, antimicrobials and lactic acids. Firstly, fungi contain a group of extracellular enzymes that facilitate the biodegradation of recalcitrant compounds such as phenolic compounds, dyes, and polyaromatic hydrocarbons (PAH), among others, through non-specific oxidation reactions [16–18]. By contrast, the bacterial cell produces target-specific enzymes for degrading contaminants [19, 20]. Fungi also have a greater resistance to inhibitory compounds than bacterial species. The hyphal growth of fungi provides a greater protection to their sensitive organelles. The larger surface area of fungi acts like an adsorption layer of extra-polysaccharide matrix, protecting them from inhibitory compounds. Moreover, fungi are eukaryotes, having considerably more genes than bacteria, which further make them more versatile in tolerating inhibitory compounds [5]. The higher number of genes in fungi imparts greater reproductive selectivity, which might result in better environmental adaptations [21].

Bacterial biomass in biological wastewater treatment has been studied for half a century and activated sludge and attached growth kinetics have been developed extensively. The kinetics of fungal processes in wastewater treatment and biomass/byproduct production are more novel and not so exhaustively developed. Thus, it is necessary to establish a better understanding of fungal systems to gain acceptance as a wastewater treatment technology.

The purpose of this review is to (1) summarize the technical literature pertaining to fungal wastewater treatment, (2) identify the unique merits and demerits of the fungal process, and (3) examine the pertinent operating constraints of the fungal process. Additionally, the review also summarizes important features related to fungal treatment not previously discussed, and outlines the future research directions.

2 Microbiology of Fungi

Fungi are eukaryotes varying in size and shape [22]. The size of fungi varies from individual cells (e.g., yeasts) to long chains of cells that stretch for miles. Fungal cells are oblong (3–8 μm × 5–15 μm) with very large trichomes containing organelles and large intracellular granules and structures. Chitin is one of the

Table 1 Characteristics of major fungal divisions [103, 104]

Division	Characteristics
Chytridiomycota	The fungi produce zoospores capable of moving on their own through a liquid medium by simple flagella
Zygomycota	The hyphae do not have one nucleus per cell, but rather have long multinucleate, haploid hyphae that comprise their mycelia. Asexual reproduction is by spores produced in stalked sporangia
Ascomycota	They contain more than 30,000 species of unicellular (yeasts) to multicellular fungi. Yeasts reproduce asexually by budding and sexually by forming a sac/ascus.
Basidiomycota	Mushrooms, toadstools, and puffballs are commonly encountered basidiomycetes. These conspicuous features of the fungi are the reproductive structures. Sexual reproduction involves the formation of basidiospores on club-shaped cells known as basidia
Deuteromycota	A group of fungi that either lack the perfect stage (i.e., sexual reproduction) or whose perfect stage is as yet undiscovered. They reproduce most frequently by conidia or conidia-like spores. Many forms of deuteromycota are pathogenic, affecting man, animals, or plants

important components of fungal cell walls. Some fungi are saprophytes (grow on dead organic matters), while others are parasites. Fungi are absorptive heterotrophs as they break down organics by secreting digestive enzymes onto the substrates and then absorbing easily accessible organic molecules. Fungal hyphae have small volumes but large surface areas, which enhance the absorptive capacity of fungi.

2.1 Broad Fungal Classification

Fungi are broadly classified as macrofungi and microfungi according to the size of their fruiting bodies. Macrofungi form fruiting bodies readily visible to the naked eye, with a diameter of at least 1 mm (e.g., mushrooms). Microfungi, on the other hand, are microscopic, having minute fruiting bodies that cannot be seen by the naked eye (e.g., *Penicillium*). Their mode of reproduction is through spore formation. Most fungal spores range from 2 to 20 μm in size and are of different shapes and colors [22]. Fungi are more fundamentally classified by their mode of reproduction (both sexual and asexual) and the nature of their multinucleate or multicellular hyphal filaments. Historically, true fungi are classified into five taxonomic divisions. The characteristics of each division are given in Table 1.

3 Composition of Fungi

Fungi, being eukaryotes, have a cellular chemistry similar to that of other eukaryotic organisms [23]. The DNA base ratio (percent guanine + cytosine) of eukaryotic cells is reported to be 38–63%; while the DNA content of fungi has

been found to be as low as 0.15–0.30% [24]. Most fungal carbohydrates are polysaccharides such as chitin, chitosan, mannan, glucan, starch, and glycogen. Chitin, a principal cell-wall component in Dikaryomycota, which includes the divisions Ascomycota and Basidiomycota and is characterized by hyphae with perforated septa, which usually occur in the dikaryotic phase), is a polymer of β-1,4 N-acetylglucosamine. Chitin is a long-chain polymer of an N-acetylglucosamine, a derivative of glucose, and is found in fungi and invertebrate animals. It is the main component of the cell walls of fungi, the exoskeletons of arthropods such as crustaceans (e.g., crabs, lobsters, and shrimps) and insects, the radulas of mollusks, and the beaks of cephalopods, including squid and octopuses. In terms of structure, chitin may be compared to the polysaccharide cellulose and, in terms of function, to the protein keratin. Chitin has also proven useful for several medical and industrial purposes. Chitosan, a deacetylated form of chitin is found along with chitin and has various medical and industrial uses as well.

Although glycogen is the main storage polymer, disaccharides such as trehalose and sugar alcohols such as mannitol are also produced in fungal cell walls. Lipids include long-chain fatty acids (such as palmitic, oleic, and linoleic acids), phospholipids, and sphingolipids [24].

There are a number of valuable fungal biochemicals that have industrial use. These, and the fungi that produce these, are outlined in Table 2.

4 Growth Requirements of Fungi

Fungi are free-living heterotrophic osmotrophs in which assimilation of digested food material takes place through the cell wall [23, 25]. Fungi absorb nutrients from the substrates on which they grow. They absorb simple, easily soluble nutrients, such as sugars through their cell walls. They excrete digestive enzymes to break down complex nutrients into simpler forms that they can absorb. Fungi derive their energy and intermediates for synthesis from oxidation of compounds through respiration and fermentation.

4.1 Carbon Source and Nutrients

Carbohydrates constitute the major carbon source. Fungi differ widely in their abilities to use different carbon sources, and the utilization efficiency of a defined carbon source may be affected by the medium composition and the culture conditions, such as pH. Various wastes such as cellulosic and lignocellulosic wastes, starch wastes, cheese whey, spent sulfite liquor (black liquor from sulfite process), molasses, and sugar beet pulp can be used as substrates for MBP/fungal byproduct production. These substrates differ in their compositions that are suitable for growing different types of fungi. For example, the white-rot fungi and brown-rot

Table 2 Fungal derived by-products and their economic importance

Product	Fungal species	Reference	Applications
Amylase	*Aspergillus niger, Aspergillus oryzae, Lentinula edodes, Neurospora crassa*	El- Zalaki and Hamza [105] Jin et al. [6] NCIM [106]	Used in brewing and fermentation industries, in the laundry industry, in the paper and food industry
Asteriquinone	*Aspergillus terreus*	NCIM [106]	Used for biomedical applications
Cellulase	*Aspergillus fumigatus, A. niger, Cladosporium sp., Fusarium sp., Myrothecium verrucaria, Phanerochaete chrysosporium, Gloeophyllum trabeum, Trichoderma reesei*	NCIM [106] Rasmussen 2006; Shrestha [90]	Used as digestive aids, for the management of flatulence, ethanol production from cellulose
Cell-wall lytic enzyme	*Trichoderma reesei, Trichoderma viride*	NCIM [106]	Used in hydrolysis
Collagenase	*Arthrobotrys conoides*	NCIM [106]	Used in medicines and ointments
Chitin	*Phycomyces blakesleeanus, Aabsidiarepens, Absidia blakesleeanus, Cunninghamella elegans*	Allan et al. [107] Knorr and Klein [108] Davoust and Hansson [109] NCIM [106]	Used to reduce serum cholesterol levels, for treating drinking water, used in medicine, cosmetics, and other biomedical applications
Chitinase	*Aspergillus flavus, A. niger, A. oryzae, Beauveria bassiana, Penicillium chrysogenum*	Allan et al. [107] Andrade et al. [110]	Used in ointments, medicines, and other clinical applications
Glucoamylase	*Mucor javanicus, Neurospora crassa, Aspergillus* sp., *Rhizopus* sp.	Manjunath et al. [111] Stone et al.[112] Tan et al. [71] Norouzian et al. [113] NCIM [106]	Used in saccharification for starch conversion and alcohol production
Lactic acid	*Rhizopus oryzae Rhizopus arrhizus*	Rosenberg and Kriofíkova [114] Mirdamadi et al. [115] Jin et al. [39] Huang et al. [116] NCIM [106]	Used to ferment milk products, used as a preservative in foods
Protease	*Acremonium chrysogenum, Penicillium roqueforti, Beauveria feline*	NCIM [106]	Used in food fermentation, used in medicine

fungi are known for their ability to degrade lignin and cellulose. The ecological group of white-rot fungi includes both 'simultaneous' and 'selective' lignin-degrading species. In the latter group, lignin degradation takes place with minimized consumption of cell-wall polysaccharides (i.e., cellulose and hemicellulose). Therefore, the degradation efficiency toward cellulose is highly diversified with white-rot fungi and is species-specific. As a consequence, special applications such as biomechanical pulping require the use of selective lignin degraders. By contrast, brown-rot fungi include most efficient cellulose-degrading species [26, 27]. The white-rot fungi have been studied for treating pulp and paper mill wastewater, cork-boiling wastewater, and other similar wastewaters [28–32]. Among different species of white-rot fungi, *Phanerochaete chrysosporium* is the most effective species in degrading lignin and cellulose, but it grows slowly and synthesizes low quantities of extracellular enzymes [33, 34].

In addition to carbohydrates, fungi also utilize lipids and fatty acids such as oleate and palmitate as their carbon source [35]. Roux-Van der Merwe et al. [36] treated oil-processing plant effluent with different fungal species. The fungi were able to utilize the edible oil as their sole carbon source. A maximum COD reduction of 98.2% was achieved using fungal species, *Cunninghamella echinulata*. In addition, *Emerisella nidulans* produced 409 mg/l of γ-linolenic acid (GLA), a high-value byproduct. The lipase-producing fungi can utilize sunflower oil as a main carbon source [37]. The fungal lipases degrade the fats and oils by converting the triacylglycerols to diacylglycerols, monoacylglycerols, free fatty acids, and glycerol. These hydrolyzed products are absorbed by the cell through simple diffusion and reassembled within the cell [36, 38].

Jin et al. [39] studied the production of lactic acid and fungal biomass from different waste streams by fungal species *R. arrhizus* and *R. oryzae*. Simultaneous saccharification and fermentation processes were studied in potato, corn, wheat and pineapple waste streams. These media had starch or sugar concentrations of approximately 20 g/l. The highest rates of lactic acid production and biomass formation were found in pineapple waste, as glucose was easily accessible for fungus consumption. The results indicated that carbon sources had some impact on the lactic acid production by *R. oryzae* and fungal biomass production of *R. arrhizus*.

The nitrogen source for fungi can be nitrates, nitrites, ammonium, or organic nitrogen substances such as yeast extract, peptone, depending on the type of fungi with a few exceptions. Many fungal species can also utilize organic nitrogen because of their ability to decompose proteins. Growth enhancing amino acids and peptides include glycine, glutamic acid, and aspartic acid, while leucine can result in poor growth [40]. A balanced fungal medium should contain about ten times more carbon than nitrogen (i.e., carbon to nitrogen [C/N] ratio = 10:1). A C/N ratio of 10:1 or less ensures high protein content, whereas a C/N ratio in excess of 50:1 favors the accumulation of alcohols, secondary metabolites, lipids, or extracellular polysaccharides [40].

A major concern in fungal wastewater treatment is nutrient requirements. There have been very few studies that examined the effect of micronutrient

supplementation on organic removal and fungal biomass production during wastewater treatment. Stevens and Gregory [41] studied the growth of *Cephalosporium eichhorniae* in potato-processing wastewater to produce MBP. It was found that 0.61 g (dry weight) of biomass and 0.3 g of crude protein per gram of carbohydrate could be produced at pH 3.75, with the addition of mono-ammonium phosphate, ferric iron, and nitrogen in the form of ammonium hydroxide. The pH was maintained using sulfuric acid. The authors reported that fungi utilized organic protein in the absence of inorganic nitrogen. When inorganic nitrogen was added, fungi preferred inorganic nitrogen to organic nitrogen.

Jasti et al. [42] operated an attached growth bioreactor with plastic composite support (PCS) tubes, with and without the addition of nutrients (ammonium bicarbonate and potassium hydrogen phosphate) for treating wet-milling corn processing wastewater. The results indicated that although the addition of nutrients improved COD removal rates and observed yields, it did not affect fungal protein production to a great extent. Rasmussen et al. [43] also found little benefit in supplementing thin stillage, left-over from dry-grind corn milling ethanol production, with any nutrients for fungal cultivation.

Kurakov and Popov [44] reported that fungi had one to four orders of magnitude greater resistance to inhibitory compounds such as herbicides and germicides e.g., atrazine, butylate, metolachlor, trifluralin, and nitrapyrin; also greater nitrate and nitrite formation ability than autotrophic nitrifying bacteria.

Wheat starch-processing wastewater was treated with *R. oligosporus* in a laboratory-scale airlift reactor with a working volume of 3.5 L [45]. Production of fungal proteins and glucoamylase was also evaluated during this study. COD removal efficiency exceeding 95% was achieved with fungal biomass production of 4.5–5.2 g/l (dry wt.) within 14 h of cultivation. The effect of nutrient supplementation on the biomass yield and COD removal of few studies are given in Table 3. Jin et al. [9] and Guimarães et al. [28] did not observe any notable improvement in biomass yield and/or COD removal with nutrient addition, while other studies found a significant improvement in the performance during fungal wastewater treatment. The effect of nutrient addition in wastewater treatment is case-specific, even depending on the specific type of food processing wastewater used. It is necessary to evaluate the effect of nutrients for a particular type of wastewater prior to fungal treatment. Many studies have not assessed the effect of combined nutrient supplementation.

Essential micronutrients for fungal growth are iron, zinc, copper, manganese, molybdenum, and either calcium or strontium. Different fungal species can have their own specific nutrient needs. For example, *A. niger* requires gallium and scandium, while other fungi require cobalt [46]. Certain fungi require vitamins in trace quantities, while others synthesize their own vitamins. Many fungi are deficient in thiamin (e.g., *Phycomyces*), biotin (e.g., *Neurospora*), riboflavin, pyridoxine, nicotinic acid, and others [24, 40]. Vitamin deficiency can be temporary. Some fungal species require additional growth factors such as inositols and heme (e.g., *Pilobolus*, *Physarum polycephalus*) [40].

Table 3 Comparison of biomass production during fungal cultivation of thin stillage in stirred bioreactors (5 L) at increasing aeration rates (n = 3)

Air flow (vvm)	Cultivation length (d)	Final biomass (g dry wt/l)
0.4	7	27 ± 3
0.8	6	30 ± 3
1.0	6	36 ± 4

4.2 Temperature

Temperature is an extremely important operational parameter which affects the growth rate of fungi. The vast majority of fungi are mesophiles, thriving at moderate temperatures of about 20–40°C. Psychrophilic fungi with an optimum temperature below 10°C occur commonly in regions covered with snow and ice during the major part of the year. There are also thermophilic fungi that grow above 45°C. Maheshwari et al. [47] have described the physiology and enzymes derived from thermophilic fungi. Temperature controls the specific growth rate, metabolism, total yield, and lag period of the fungi.

Temperature affects growth rate, metabolism, nutritional requirements, regulation mechanisms of enzymatic reactions, and cell permeability in a microorganism. The structure and composition of cytoplasmic membranes in cells are also altered by temperature that determines the substrate utilization rate of fungi [48].

Thermophilic fungi require a minimum temperature of growth at or above 20°C and a maximum temperature of growth extending up to 60 or 62°C. The hypothesis behind using thermophilic fungi is that a higher growth rate will achieve a better degradation rate [47]. The thermophilic fungi break down the soluble-protein fractions at a rate twice that of mesophilic fungi.

4.3 pH

The effects of pH on fungal metabolism include availability of metal ions, cell permeability, and enzymatic activity. The optimum pH for most fungi is in the acidic range of pH < 5.0. Lower pH increases iron availability, while higher pH increases enzymatic activity. It is possible to have two optimum values in a pH growth curve for fungi [46].

The biochemical and enzymatic reactions in any biological system vary with pH. Numerous studies were conducted to determine the optimum pH for different fungal species [41, 49–52]. In these studies, pH was optimized based on their research objectives (e.g., organic removal or fungal biomass/byproduct production). Van der Westhuizen and Pretorius [31] reported that the yield coefficients of *A. fumigatus* were 0.44, 0.39, and 0.38 g/g COD at pH 5.0, 5.5, and 6.1, respectively. As pH increased, the number of opportunistic bacteria under non-aseptic conditions increased from 1×10^6 to 30×10^6 colony-forming units (CFU)/ml. Similar results were also reported by Jasti et al. [42] while using *R. oligosporus* to

treat wet corn-milling wastewater. The bacterial biomass concentration was found to be greater at a pH of 4.5 than at pH of 4.0. Thus, it could be concluded that the pH affects growth rate as well as the microbial competition in a mixed culture.

The morphology of the fungi is also affected by the pH. The morphological changes attributed to pH variation were from fluffy to clumpy and compact fungal pellets [53, 54]. Jin et al. [45] investigated the morphological characteristics of three different strains of *A. oryzae* (e.g., DAR 1,679, 3,699, and 3,863) and their yields at varying pH (3.0–6.0) conditions while treating wheat starch-processing wastewater. The fungal morphology as well as the yield coefficients differed to a great extent at different pH conditions. Compact pellet formation occurs mainly at pH values 4–6. The morphology of fungi, in turn, affects the viscosity of the medium, thereby affecting the oxygen transfer efficiency and mixing in the biological system. Van Suijdam and Metz [55] described the influence of various parameters (chemical factors: carbon dioxide, substrate, pH; physical factors: shear, temperature, pressure) on the morphology of filamentous fungi.

4.4 Oxygen

Most fungi are obligate aerobes, requiring molecular oxygen for their growth. Therefore, fungi are usually found growing on or near the surface of the substrate. Some fungi are facultative anaerobes, which survive in oxygen-limited environments, including sewage sludge and polluted waters [56]. Utilization of carbon and nitrogen may be affected by oxygen availability. Insufficient oxygen supply increases the nutritional demand and thereby decreases fungal growth [46]. Oxygen supply is one of the most important operational costs in fungal cultivation, so proper design is a critical issue, which was an important factor in embarking on pilot-scale studies.

5 Ethanol Plant Co-product Beneficiation

Fuel ethanol production in the US is booming with production up to 863,000 barrels/day, or more than 13 billion gallons per year reached in 2010 [57]. This is more than 10% of the US gasoline needs. Biofuels, such as corn ethanol, help alleviate oil dependence and improve energy security, while boosting the rural economy and reducing greenhouse gas emissions. The corn-ethanol industry has advanced in efficiency, but still has considerable room for improvement.

Reducing energy inputs, creating value-added coproducts from stillage, and recycling water are important to sustainable corn ethanol production. Innovations for improved sustainability help to contest recent ethanol issues, such as indirect land use changes and food versus fuel, which affect public perception of corn-ethanol. Almost 90% of all ethanol in the US is produced by dry-grind ethanol plants [58]. Most conventional dry-grind corn-ethanol plants use on average 3.5 gal of fresh water [59] and generate 5–6 gal stillage per gal ethanol after

distillation, of which up to half is recycled directly as backset [60]. The ethanol-to-stillage ratio in the beer from a finished fermentation is limited by ethanol toxicity to the yeast. The profitability of dry-grind ethanol production depends on the sale of both the ethanol and the coproducts from stillage. Recycling water is important as there are limits to supply, and the ethanol plants are not permitted to discharge processing water.

5.1 Ethanol Plant Co-product Beneficiation with Fungi

Dry-grind corn-ethanol plants, the backbone of a rapidly expanding biofuel industry, generate copious amounts of stillage, the leftovers from fermentation followed by distillation. Most thin stillage is currently concentrated by flash evaporation–an energy-intensive process–blended with distillers grains, and dried to produce distillers dried grains with solubles (DDGS). Thin stillage is generated in pasteurized condition and is rich in nutrients, with a chemical oxygen demand (COD) up to 100 g/l. The initial pH of 4, high organic content, and a C:N:P ratio of about 40:5:1 make it an ideal feedstock for fungal cultivation.

Dry-grind processing produces ethanol from corn by milling, hydrolyzing with enzymes (with or without cooking), fermentation, and distillation. Approximately one-third of the corn is converted to ethanol, one-third to carbon dioxide, and one-third remains as dissolved and suspended organics in the stillage. Most suspended solids—wet distillers grains—are removed by centrifugation. The liquid centrate—thin stillage—contains mainly dissolved organic materials (solubles) of 75–100 g/l as COD [61, 62]. Up to half of the thin stillage is recycled directly as process water, and the remainder is evaporated to produce a syrup of 30% solids [63], requiring a substantial part of plant energy inputs. The syrup is blended with wet grains and dried to produce DDGS. Dry-grind corn-ethanol plants produced 34.6 million metric tons of distillers dried grains in 2010, a 150% increase from the 14.6 million metric tons in 2007 [64].

Distillers grains are sold primarily as livestock feed for cattle and dairy. The low content of digestible amino acids limits its use for non-ruminants. Only 11 and 5% of distillers grains were consumed by swine and poultry, respectively, in 2007, in spite of higher proportions of hog and chicken production in the US Corn Belt. The syrup has suffered a decline in demand as it is not nutritionally expedient to include syrup; it is sold for very low prices and is even given away [65].

The low initial pH of 4 and high organic content make thin stillage an ideal feedstock for fungal cultivation. Fungal treatment of thin stillage has the potential to recover water and enzymes for in-plant reuse and to produce a high-quality animal feed (distillers dried grains with fungal protein or fungal protein only). The fungal coproduct could command an increased market value, while improving profits and minimizing environmental impacts. Based on 6 gal stillage per gal ethanol and recycling 50% as backset, there is 3 gal thin stillage available for fungal treatment.

The use of fungi is advantageous as they produce a wide array of biochemicals and enzymes, which tend to be more effective in degrading complex carbohydrates than bacteria ([8, 23]). The food-grade fungus *R. microsporus* var. *oligosporus* (shortened *R. oligosporus*) produces numerous enzymes, including β-glucosi-dase, glucoamylase, lipase, phytases, and chitinases [53, 66–70]. *Rhizopus* species are also known to produce α-amylase [6]. Under aseptic conditions in a nutrient medium, *R. oligosporus* was reported to produce a 3% yield of chitosan [71], as well as 4 and 40% yields of lysine and protein, respectively [72]. *R. oligosporus* was successfully cultivated on wheat milling and corn wet-milling streams, achieving significant reductions in COD of up to 80–90% [6, 7, 9, 39, 42, 45, 49, 53, 73, 74–80]. The filamentous nature of fungal mycelia and potential for pellet formation aid in the recovery of fungal biomass [9, 14].

This research won a 2008 American Academy of Environmental Engineers Grand Prize for University Research, 2008 R&D 100 Award, and 2008 International Water Association Project Innovation Award and is the first study of fungal cultivation on dry-grind corn-ethanol stillage (patents pending).

Based on the utility of fungi and the readily available low-cost substrate, bench- (5 L) and small pilot-scale (50 L) experiments were conducted to evaluate fungal treatment of thin stillage for fungal biomass production and organics removal to obtain recyclable effluent for in-plant use. Aeration rates in stirred bioreactors varied from 0.2 to 1.0 L air/l working volume/min (vvm). Feed stillage and bioreactor samples were analyzed to determine the removal of total and soluble COD (TCOD, SCOD), total and volatile suspended solids (TSS, VSS), glycerol, and lactic and acetic acids, critical for recycling the effluent as process water. Fungal biomass production was quantified, and samples were analyzed for protein and amino acid contents.

6 Laboratory-Scale Fungal Cultivation on Ethanol Plant Thin Stillage

Bench-scale experiments with thin stillage, filled with small fungal pellets within 2 d of spore inoculation (Fig. 1) [43]. Mycelia continued to grow in suspension as pellets. Fungal mycelia and sporangiospores were observed under the microscope on days 1 and 4, respectively (Fig. 2).

6.1 Organic (COD) Removal

The total and soluble organic content of fresh thin stillage batches, as measured by COD, ranged from 81 to 98 g TCOD/l and 49 to 60 g SCOD/l, respectively. Reductions in SCOD tended to improve with increasing aeration rates up to

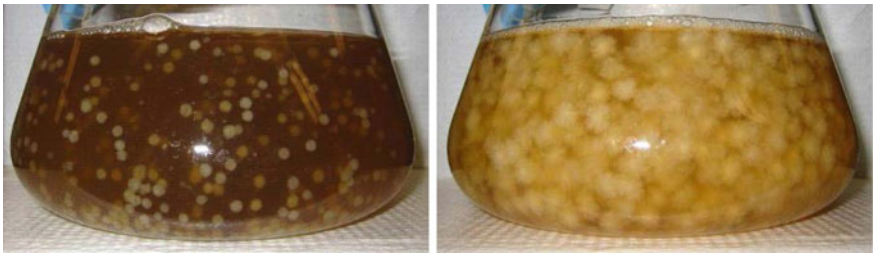

Fig. 1 Fungal pellet growth in thin stillage

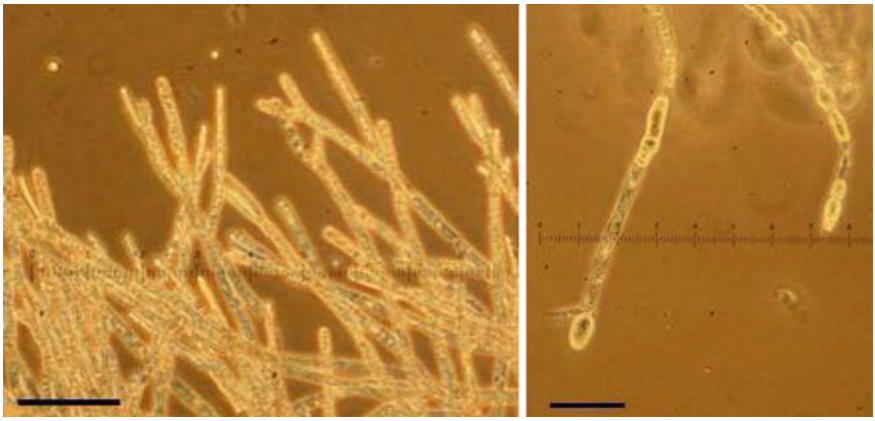

Fig. 2 Microscope photos showing *R. oligosporus* growth

0.8 vvm in bench-top stirred bioreactors (Fig. 3). The SCOD was reduced by 17, 36, and 46% in 5 d with aeration rates of 0.2, 0.4, and 0.8 vvm, respectively. Increasing the aeration rate to 1.0 vvm had a slightly lower SCOD removal in 5 d (42%) than 1 obtained with 0.8 vvm (46%). This outcome may be attributed to the concentrating effect of rapid reduction in liquid volume with 1.0-vvm aeration, due to evaporation and suspended solids accumulating on the vessel wall blown out by excessive bubbles. The DO level with 0.8-vvm aeration, the optimal rate for SCOD reduction in 5-L bioreactors, dropped rapidly from 100 to 75% of saturation in 1 d and to 3% in 2 d (Fig. 4). The DO remained at ca. 4% of saturation from day 2 to day 5. This low DO was due to the rapid oxygen consumption by the fungal biomass.

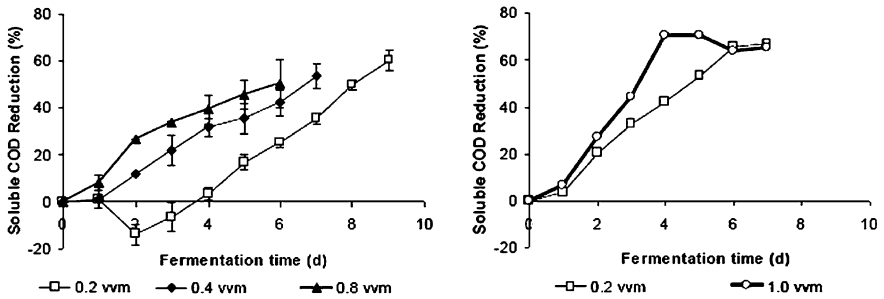

Fig. 3 Removal of COD as a function of aeration rate

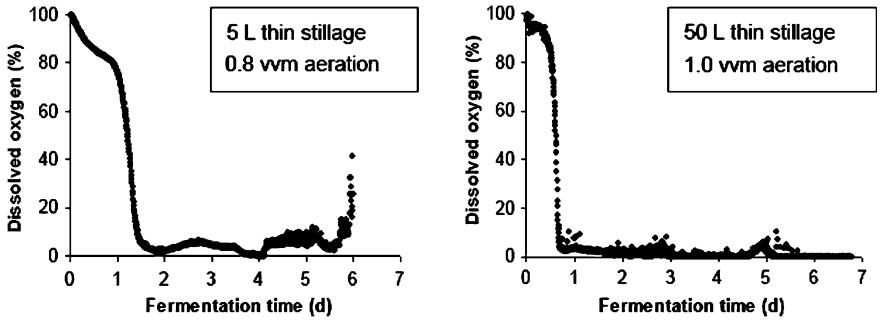

Fig. 4 Dissolved oxygen levels during fungal growth in thin stillage

6.2 Suspended Solids Removal

The initial suspended solids were between 20 and 30 g/l in fresh thin stillage batches. Total and volatile suspended solids contents were similar in all experimental samples, indicating low levels of fixed, inorganic suspended solids. Reductions in suspended solids in daily bioreactor samples differed based on the aeration rate (Fig. 5). Gradual removal of suspended solids was observed with 0.2-vvm aeration from day 0 through day 4 (up to 39%), in part due to accumulation on the bioreactor wall above the liquid level and between the probes and vessel wall. The suspended solids concentration decreased rapidly after day 4 reaching 89 and 99% removals by days 5 and 6, respectively; this coincided with the liquid clarification one observed on day 5 (TCOD and SCOD became equal). Thin stillage particles were removed by attachment to mycelia and by biological mineralization.

Liquid collected through the effluent port was well-clarified, as low as 20 mg/l suspended solids, with a yellow tint (Fig. 6). Solids separation before returning the water to the corn fermentation process is very important to avoid the build-up of

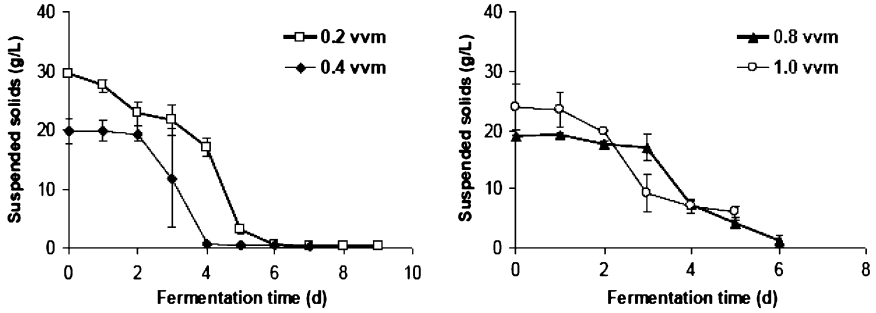

Fig. 5 Removal of suspended solids during fungal fermentation

Fig. 6 Reactor effluent

non-biodegradable substances. The fungal process effluent could potentially be recycled with minimal further treatment. An increased aeration rate of 0.4 vvm resulted in more rapid clarification of the bioreactor samples on days 3 and 4 (Fig. 5). The average solids removals with 0.4-vvm aeration were 45 and 97% in 3 and 4 d, respectively.

6.3 Organic Acids and Glycerol Removal

Initial lactic and acetic acids concentrations in fresh thin stillage ranged from 1.5 to 3.1 g/l and 1.0 to 1.5 g/l, respectively. The glycerol content in thin stillage was high, between 12.8 and 15.9 g/l. Organic acid production is primarily from

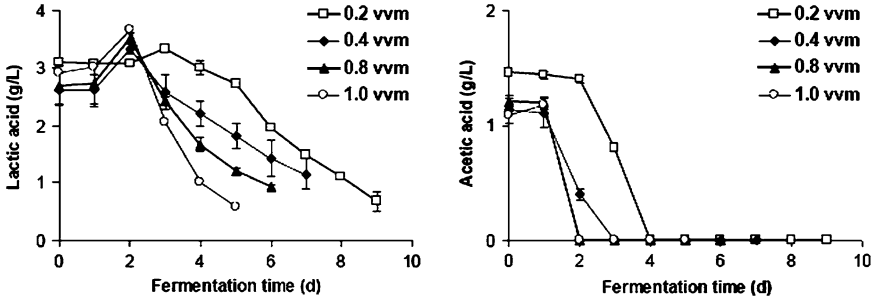

Fig. 7 Lactic and acetic acid removal from thin stillage by *R. oligosporus*

bacterial contamination during yeast fermentation in the ethanol plant. Glycerol accumulation is a byproduct of yeast fermentation under stressed conditions (Walker [81]). In bench- and pilot-scale bioreactors, fungal removal of lactic acid, acetic acid, and glycerol tended to improve with increasing aeration rates. Lactic acid contents in bench-top bioreactor samples increased during the first 2 d of fungal cultivation (Fig. 7). This trend may indicate that the fungus was producing lactic acid initially.

Several *Rhizopus* strains are known to produce lactic acid in submerged cultures [82]. From day 2 onward, lactic acid concentrations decreased, with reductions of 14, 31, 54, and 71% with aeration of 0.2, 0.4, 0.8, and 1.0 vvm, respectively, by day 8. Fermentation of soybeans with *R. oligosporus* for tempeh production confirms that this fungal species is able to degrade lactic acid [83]. Fungal cultivation of settled thin-stillage supernatant improved lactic acid biodegradation, with 60% removal in 5 d with 0.8-vvm aeration (as compared to 54% with thin stillage particles). Lactic acid reductions were substantially higher in the 50-L bioreactor, reaching 100% in 6 d with 0.2-vvm aeration and in 4 d with 1.0-vvm aeration, which may be related to better DO transfer. Acetic acid removals also improved with increasing aeration rates (Fig. 7). Complete removal was achieved in 4 d with aeration of 0.2 vvm, in 3 d with 0.4 vvm, and in 2 d with 0.8 and 1.0 vvm. Similar results were obtained using the settled thin stillage supernatant with 100% removal in 2 d with 0.8-vvm aeration. In the 50-L fungal cultivations, the acetic acid removal was more rapid with 100% removal by day 2 with 0.2-vvm aeration and by day 1 with 1.0-vvm aeration.

Glycerol biodegradation in the thin stillage began 2 d after fungal inoculation (Fig. 8). This delay may be associated with the time required for the fungal culture to grow from spores. Reductions in glycerol concentrations by 1 day 5 reached 11% with aeration of 0.2 vvm, 22% with 0.4 and 0.8 vvm, and 45% with 1.0 vvm.

Glycerol reduction in 50-L bioreactors was significantly better with 100% removal achieved in 6 d with 0.2-vvm aeration and 5 d with 1.0-vvm aeration, which again suggests better oxygen transfer in the 50-L compared to the 5-L experiments.

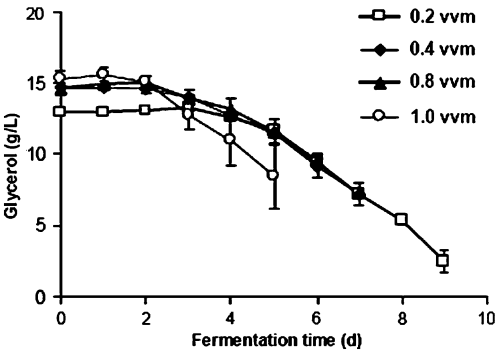

Fig. 8 Glycerol removal by *R. oligosporus*

6.4 Fungal Biomass Production

Quantifying fungal biomass production was complicated by the removal of thin stillage solids via attachment to the vessel wall above the liquid level and to the fungal mycelia. Thin stillage particles attached to the surface of the fungal biomass and within it, particularly inside the mycelia on the agitator blades. The final biomass weights provided in Table 3 include the freeze-dried fungal biomass and attached thin stillage solids, which were collected at the end of the experiments. The harvested biomass tended to increase with increasing aeration rates.

In the 50-L cultivation with 1.0-vvm aeration, more of the fungal mycelia and thin stillage solids remained in suspension. The production of 28 g dry biomass/l thin stillage was lower than obtained in the bench-top bioreactors, in part because centrifugation did not recover as much of the thin stillage solids as attachment to fungal mycelia.

6.5 Fungal Protein and Amino Acids

The average crude protein content of *R. microsporus* mycelia grown in YM broth for inoculation of the 50-L cultivations was 39% (w/w). The fungal biomass cultivated on settled thin-stillage supernatant (no suspended solids) had up to 43% (w/w) crude protein. This fungal protein could be fed to the nonruminants, e.g., swine and poultry.

Lysine, methionine, tryptophan, and threonine are the main amino acids of importance for nonruminants [84]. Lysine and methionine are usually the first-limiting amino acids in corn-soybean meal diets for swine and poultry, respectively. Based on the amino acid data in Table 4, the dried fungal biomass had almost two-thirds the lysine and over 2.5 times the methionine contents of soybean meal (% as-fed basis). The fungal lysine, threonine, and tryptophan contents, however, were all comparable to soybean meal on a percent of protein basis (Fig. 9). Biomass protein contents and digestibility depend on harvesting,

Table 4 Amino acid composition of biomass on as-fed basis (10% moisture) compared with soybean meal, corn, and DDGS

Amino acid (%)	Fungal biomass[a] (%)	Soybean meal[b] (%)	Corn[b] (%)	DDGS[b]
Lysine	1.8	3.0	0.3	0.6
Methionine	1.8	0.7	0.2	0.5
Tryptophan	0.3	0.7	0.1	0.3
Threonine	1.5	1.9	0.3	1.0

[a] Freeze-dried fungal biomass product includes enmeshed stillage solids
[b] Data from National Research Center [117]

dewatering, and drying methods, as well as amounts of enmeshed stillage solids. Improved dewatering should increase the protein fraction 22 (%) of the fungal biomass and, consequently, the lysine content (% as-fed). Water reclamation and fungal protein content may be enhanced by supplementing thin stillage with readily bioavailable inorganic nitrogen sources.

6.6 Conclusion on Laboratory Studies

Fungal treatment of thin stillage in corn-ethanol plants is an innovative technology to reclaim water, save energy and potentially enzymes, and produce an additional valuable coproduct. Recycling fungal-treated water directly could provide substantial energy savings by avoiding the current practice of evaporating and condensing water from thin stillage. The high-protein fungal biomass produced could be fed to nonruminants. The fungal biomass could also be used as raw material for the extraction of valuable biochemicals, such as chitosan. Fungal cultivation of thin stillage has the potential to make ethanol production more energy efficient and more sustainable, to reduce costs, and to produce an additional value-added coproduct.

Many practical aspects and equipment development could not be done on laboratory scale. The laboratory results encouraged a further study on pilot-scale and this is described below.

7 Pilot-Scale Research and Development

A fungal treatment process for thin stillage has the following merits

(1) Energy savings by avoiding stillage evaporation,
(2) Recovery of protein-rich fungal biomass, and
(3) Potential for water recycling of treated effluent.

Solids separation and removal of organic materials are important for recycling the effluent as process water. This research focused on investigating the cultivation of the food-grade fungus *R. microsporus* on thin stillage and the potential for water recycling on a 1,400 L pilot plant.

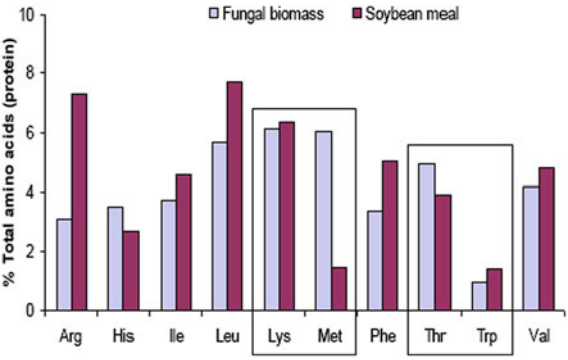

Fig. 9 Comparison of ten amino acids in fungal biomass with soybean meal

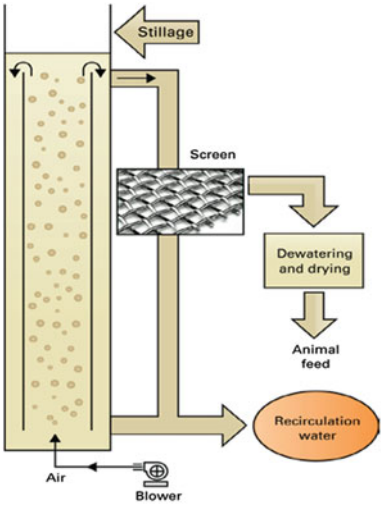

Fig. 10 Pilot plant concept

7.1 Reactor Development

Cultivation of the filamentous fungus, *R. microsporus* required construction of a deep airlift reactor to ensure high-rate aeration for rapid fungal growth and pellet morphology. The concept is shown in Fig. 10.

The 1,400 L airlift reactor consists of a 6 m high cylindrical tank of 600 mm, with a draft tube with a diameter of 450 mm inside the tank to a height of 4.5 m to make it possible for the liquid to circulate by flowing upward through the draft tube and flow back through the annulus. Aeration of thin stillage, with fungal inoculum, is effected within the inner part using a blower with an array of seven porous diffusers. Aeration of thin stillage, with fungal inoculum, is effected within the inner part using a blower with an array of seven porous diffusers.

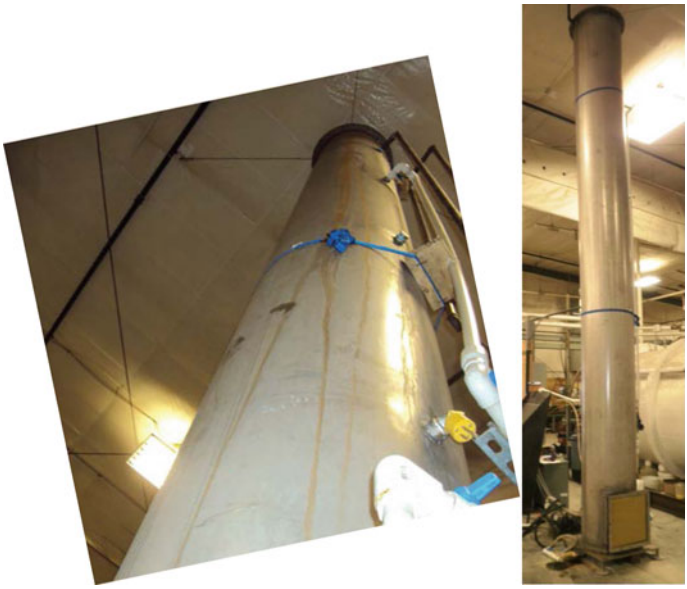

Fig. 11 Photos of the pilot airlift fungal cultivation reactor

The aerated liquid moves upward along with the bubbles, due to the lower density of the bubble-filled liquid compared with that in the annulus. The bubbles are released at the top and the liquid recirculates downward in the annulus. The aeration rate is varied to establish operational procedures for full-scale implementation. The oxygen demand of the fungi is actually the main determining factor for aeration needs and aeration requires the largest power input required for fungal cultivation. Figure 11 shows photos of the airlift reactor and Fig. 12 one of the porous diffusers used.

7.1.1 Pre-propagation Facilities

The pilot plant has been designed and equipped with a heating system at the bottom for fungal propagation of about 10% of the main volume for better startup conditions. This system was discontinued because of leaks and a culture is propagated in one or both of two specially constructed 50-L pre-propagation reactors. In each case, a pre-inoculum is prepared in 5-L shaker flasks. The ultimate aim is to operate in continuous mode. The pre-propagation set-up is shown in Fig. 13.

7.1.2 Harvesting Screen and Dewatering

The harvesting screen was built with a recirculation sump and level control. The harvesting screen in shown in Fig. 14

Fig. 12 Photo of one of the porous diffusers

Fig. 13 Pre-culturing set-up for preparing pilot plant inoculum

The biomass is dewatered by retention on the primary screen, followed by transfer to secondary horizontal screen shown in Fig. 15 and final dewatering in polyethylene woven sacks.

Fig. 14 Primary fungal harvesting also showing detail of screen openings

Fig. 15 Secondary screen dewatering equipment

Fig. 16 Cellencor microwave dryer showing details of feeding the fungal biomass and the dried product emerging from the drying tunnel

7.1.3 Drying

The dewatered biomass is passed through a Cellencor microwave tunnel dryer. This is shown in Fig. 16.

7.2 Results

Fungi grew prolifically, yielding on average 20 kg of biomass per batch run from 1,300 to 1,350 L of thin stillage. The fungal pellets take 14–20 h to develop, and the aeration requirements were much lower than the lab results. Removal of COD, suspended solids, glycerol, and organic acids, critical for stillage recycling,

reached 60–80%. It was ascertained that there is no need for pH control with enough acidity already in the thin stillage.

The pellets are easy to harvest using the curved inclined screen and the harvested product from the primary screen contains 10–12% solids. Secondary screening, followed by gravity draining in woven polyethylene bags overnight removes about half of the remaining water, to reach a solid concentration of 22%.

The biomass was dried to 8% moisture using the Cellencor microwave drying tunnel.

The fungal biomass is high in lysine and nutraceuticals chitin/chitosan, enhancing its potential as a nutritionally beneficial livestock feed. The fungus could be co-fed with DDG to monogastrics: swine and poultry, thereby adding value to another coproduct from the ethanol industry.

Continuous operation has remained elusive due to the propensity of yeast co-cultures to develop. These are difficult to harvest. The current answer to this is regular disinfection with chlorine and steam.

The dried product and a dish prepared from the fungi for human consumption are shown in Fig. 17.

7.3 Other Uses for Fungi

Other uses of dried fungal biomass have been studied by Ozsoy et al. [85], and Ozsoy and van Leeuwen [86, 87]. The general finding was that dried fungal biomass is a good adsorbent for removing metal ions from water. This could have application in the detoxification of industrial wastewaters. Further application could be the extraction of fine chemicals from the fungal biomass, including chitin, chitosan and glucosamine, and other biochemicals.

7.4 Other Fungal Processes for Byproduct Beneficiation

Processing crops inevitably leaves huge amounts of residues of fibrous materials. These are mainly lignocellulosic materials that could be converted to more biofuels [34, 88, 89]. Various wood-rot fungi can be used to release the bonds between cellulose, hemicellulose, and lignin to liberate cellulose for further degradation into constituent sugars. These sugars could then be fermented to either produce more ethanol ([34, 88, 90–96]) or could be fermented to produce triglycerides/stored fats using oleaginous fungal strains for the production of biodiesel feedstock (Karki et al. [97]). Mitra et al. [98] designed and optimized the fermentation of an oleaginous fungal strain, *Mucor circinelloides* on thin stillage (TS). This project enabled corn-ethanol co-product beneficiation with concomitant production of fungal oil-based biodiesel feedstock. Substantial increases in oil yield were found when *M. circinelloides* was grown on TS as compared to a

Fig. 17 Dried fungal biomass for animal feed and a dish prepared from fungal biomass

standard fungal nutrient medium, YM broth (yeast mold broth). In another study by Roux-Van der Merwe et al. [36], effluent from an oil-processing plant was used as the fermentation feedstock for growing fungal strains for the production of high-value gamma linolenic acid (GLA). The results of this study indicated that fungal treatment reduced the COD (chemical oxygen demand) of the effluent by almost 98% while producing 373 mg GLA per liter of the waste stream. Falanghe et al. [99] used soybean whey, a coproduct from the soybean processing industry to grow fungal strains for the production of nutrient-rich fungal mycelial protein. The group obtained almost 4–6 g of mycelial protein per liter of whey. Utilization of and value addition to soybean whey benefitted whey disposal as well as lead to production of animal feed with a high protein content.

Fungi produce large quantities of organic compounds, including acids such as itaconic acids, oxalic acids, lactic acid, pigments, e.g., β-carotene and astaxanthin and aromatic alcohols, e.g., resorcinol and phenol [46]. More importantly, chitin, as a major cell-wall constituent in fungi, is a polymer of glucosamine, a very important nutraceutical in the US and a prescribed medicine in most other countries, also used for pets as a relief for arthritis symptom alleviation and fungi, rather than the current main source, i.e., crustacean exoskeletons could be more readily recoverable supply of glucosamine.

Chitosan is another high-value product with several practical uses and an increasing market demand in the cosmetics, pharmaceuticals, agricultural, and food industries. Chitosan can be produced from fungal biomass grown on industrial co-products. A group of researchers led by Yokoi et al. [100] utilized a *shochu* distillery wastewater as culture medium for the cultivation of *Absidia* and *Gongronella* sp. They obtained almost 356 mg of chitosan per liter of wastewater. Another study by Suntornsuk et al. [101] established a beneficiation process for food-processing by-products specifically legume-processing wastes using fungal fermentation. Chitosan yields obtained from *R. oryzae* on soybean residue was 4.3 g/kg substrate. Other high-value products like polyunsaturated fatty acids

(PUFAs), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) which have established beneficial effects on human health have been produced from fungi using food processing by-products and crude soybean oil as the fungal fermentation feedstock. EPA has also been reported to be produced during value-addition to a dairy industry based by-product stream, sweet whey permeate (O'Brien et al. [102]). The filamentous fungus *Pythium irregulare* grown on the whey gave a high specific productivity of ~25 mg EPA/g dry biomass leading to amelioration of a significant disposal problem of the dairy industry.

7.5 Discussion

A novel, low-energy fungal process was developed that remediates dry-grind corn-ethanol thin stillage with simultaneous generation of nutritious fungal biomass. The process eliminates the need for evaporation of the water to recover the dissolved organics by conversion to microbes that are readily removed by screening and dewatered further for the production of animal feed, which is to be evaluated in the next phase by feeding piglets.

Rhizopus microsporus was able to utilize most stillage organic substances, which was not inhibited and it enmeshed remaining suspended solids. These are important properties in the economical operation of a full-scale fungal process.

7.5.1 Socio-economic Impacts

The US ethanol industry consumes 35 billion gallons water per year to produce 12 billion gallons ethanol. Water consumption could be reduced by at least 10 billion gallons if all dry-grind ethanol plants recovered water by this process. The current evaporation process costs about $0.04/gal ethanol produced at current natural gas prices. Energy savings from eliminating stillage evaporation could save $400 million/year nationwide. Enzymes recycled with fungal-treated water from stillage could further save $60 million in value per year. The potential revenue from high-quality livestock feed production, along with expanding the DDG market, is expected to be worth another $400 million/year. Preliminary cost estimates of implementing a fungal stillage treatment process indicate that the amortization and operational costs of the fungal fermentors and separation equipment amount to about 50% of the savings and additional income. The value of the energy savings, fungal biomass as livestock feed, and enzymes produced is estimated at 20 ¢/gal ethanol, with half of that required in alternative processing costs. Considering all these cost figures, it is estimated that the value added for the ethanol industry would be $0.6 billion/year. The fungal biomass is also an ideal source of the nutraceuticals chitin/chitosan, constituting 5–9% of the biomass, traditionally obtained from crustaceans at a cost of about $8,000 per ton. These have been demonstrated to improve animal growth and health and eliminate the need for

antibiotics. This would lead to healthier meat products. Industrial implementation of fungal treatment of stillage will lead to job creation and improved rural prosperity.

7.5.2 Research Outputs

The concept has two patents pending and publications have been accepted in top journals in the field. The fungal work was awarded the Grand Prize for University Research by the American Academy of Environmental Engineers in 2008, 2009 and 2011 and won R&D 100 awards in 2008 and 2009.

References

1. Metcalf and Eddy (2003) In: Tchobanoglous G, Burton FL, Stensel HD (eds) Wastewater engineering: treatment and reuse, 4th edn. McGraw Hill, New York
2. Weemaes MPJ, Verstraete WH (1998) Evaluation of current wet sludge disintegration techniques. J Chem Technol Biotechnol 73:83–92
3. Canales A, Pareilleux A, Rols JL, Goma G, Huyard A (1994) Decreased sludge production strategy for domestic treatment. Water Sci Technol 30:97–116
4. Barbesgaard P, Heldt-Hansen HP, Diterichsen B (1992) On the safety of aspergillus oryzae: a review. Appl Microbiol Biotechnol 36:569–572
5. Guest RK, Smith DW (2002) A potential new role for fungi in a wastewater MBR biological nitrogen reduction system. J Environ Eng Sci 1:433–437
6. Jin B, Van Leeuwen J, Patel B, Yu Q (1998) Utilization of starch processing wastewater for production of microbial biomass protein and fungal α-amylase by aspergillus oryzae. Bioresour Technol 66:201–206
7. Jin B, Van Leeuwen J, Yu Q, Patel B (1999) Screening and selection of microfungi for microbial biomass protein production and water reclamation from starch processing wastewater. J Chem Technol Biotechnol 74:106–110
8. Van Leeuwen J, Hu Z, Yi TW, Pometto AL III, Jin B (2003) Kinetic model for selective cultivation of microfungi in a microscreen process for food processing wastewater treatment and biomass production. Acta Biotechnol 23:289–300
9. Jin B, Van Leeuwen J, Patel B, Doelle HW, Yu Q (1999) Production of fungal protein and glucoamylase by Rhizopus oligosporus from starch processing wastewater. Process Biochem 34:59–65
10. Zheng S, Yang M, Yang Z (2005) Biomass production of yeast isolate from salad oil manufacturing wastewater. Bioresour Technol 96:1183–1187
11. Bergmann FW, Abe J, Hizukuri S (1988) Selection of microorganisms which produce raw-starch degrading enzymes. Appl Microbiol Biotechnol 27:443–446
12. Gonzalez MP, Siso MIG, Murado MA, Pastrana L, Montemayor MI, Miron J (1992) Depuration and valuation of mussel-processing wastes. Characterization of amylolytic postincubates from different species grown on an effluent. Bioresour Technol 42:133–140
13. Satyawali Y, Balakrishnan M (2008) Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: a review. J Environ Manage 86:481–497
14. Nigam P (1994) Process selection for protein-enrichment: fermentation of the sugar industry byproducts molasses and sugar beet pulp. Process Biochem 29:337–342
15. Adams RL (2001) Mycotoxins. http://www.moldreporter.org/vol1no3/mycotoxins. Accessed 23 Feb 2006

16. D'Annibale A, Ricci M, Quaratino D, Federici F, Fenice M (2004) Panus tigrinus efficiently removes phenols, color and organic load from olive-mill wastewater. Res Microbiol 155: 596–603
17. Giraud F, Guiraud P, Kadri M, Blake G, Steiman R (2001) Biodegradation of anthracene and fluoranthene by fungi isolated from an experimental constructed wetland for wastewater treatment. Water Res 35:4126–4136
18. Jaouani A, Guillén F, Penninckx MJ, Martínez AT, Martínez MJ (2005) Role of Pycnoporus coccineus laccase in the degradation of aromatic compounds in olive oil mill wastewater. Enzym Microb Technol 36:478–486
19. Chróst RJ, Siuda W (2002) Ecology of microbial enzymes in lake ecosystems. Enzymes in the environment: activity ecology and applications, vol 640. CRC Press, New York, pp 35–72
20. Riser-Roberts E (1998) Remediation of petroleum contaminated soils: biological, physical, and chemical processes. CRC Press, Boca Raton
21. Bennett JW, Lasure LL (1991) More gene manipulations in fungi. Academic Press, San Diego
22. Gravesen S, Frisvad JC, Samson RA (1994) Microfungi. Munksgaard Publishers, Copenhagen
23. Sankaran S, Khanal SK, Jasti N, Jin B, Pometto AL III, van Leeuwen J (2010) Use of filamentous fungi for wastewater treatment and production of high value fungal by-products: a review. Crit Rev Environ. Sci Biotech 40(5):400–449
24. Kendrick B (2000) The fifth kingdom. Fungal Physiology, 3rd edn. Focus publishing, Newburyport, pp 142–158
25. Dick MW (1997) Fungi, flagella and phylogeny. Mycol Res 101:385–394
26. Tanesaka E, Masuda H, Kinugawa K (1993) Wood degrading ability of basidiomycetes that are wood decomposers, litter decomposers, or mycorrhizal symbionts. Mycologia 85:347–354
27. Tuomela M, Vikman M, Hatakka A, Itävaara M (2000) Biodegradation of lignin in a compost environment: a review. Bioresour Technol 72:169–183
28. Guimarães C, Porto P, Oliveira R, Mota M (2005) Continuous decolourization of a sugar refinery wastewater in a modified rotating biological contactor with Phanerochaete chrysosporium immobilized on polyurethane foam disks. Process Biochem 40:535–540
29. Mendonca E, Pereira P, Martins A, Anselma AM (2004) Fungal biodegradation and detoxification of cork boiling wastewaters. Eng Life Sci 4:144–149
30. Raghukumar C, Mohandass C, Kamat C, Shailaja MS (2004) Simultaneous detoxification and decolorization of molasses spent wash by the immobilized white-rot fungus Flavodon flavus isolated from a marine habitat. Enzym Microbiol Technol 35:197–202
31. Van der Westhuizen TH, Pretorius WA (1998) Use of filamentous fungi for the purification of industrial effluents, WRC report No. 535/l/98, Pretoria, South Africa
32. Wu J, Xiao YZ, Yu HQ (2005) Degradation of lignin in pulp mill wastewaters by white-rot fungi on biofilm. Bioresour Technol 96:1357–1363
33. Friedrich J (1987) Mixed culture of Aspergillus awamori and Trichoderma reesei for bioconversion of apple distillery waste. Appl Microbiol Biotechnol 26:299–303
34. Shrestha P, Rasmussen ML, Khanal SK, Pometto AL III, van Leeuwen J (2008) Saccharification of corn fiber by Phanerochaete chrysosporium in solid-state fermentation and subsequent fermentation of hydrolysate into ethanol. J Agric Food Saf 56(11):3918–3924
35. Walker GM, White NA (2005) Introduction to fungal physiology. In: Kavanagh K (ed) Fungi: biology and applications. Wiley, West Sussex, pp 1–34
36. Roux-Van der Merwe MP, Badenhorst J, Britz TJ (2005) Fungal treatment of an edible-oil-containing industrial effluent. World J Microbiol Biotechnol 21:947–953
37. Ratledge C (1989) Biotechnology of oils and fats. Microbial lipids, vol 2. Academic Press, San Diego, pp 567–688
38. Finnerty WR (1989) Microbial lipid metabolism. Microbial lipids, vol 2. Academic Press, San Diego, pp 525–566
39. Jin B, Tin P, Ma Y, Zhao L (2005) Production of lactic acid and fungal biomass by rhizopus fungi from food processing waste streams. J Ind Microbiol Biotechnol 32:678–686

40. Carlile MJ, Watkinson SC (1994) The fungi. Academic Press, New York
41. Stevens CA, Gregory KF (1987) Production of microbial biomass protein from potato processing wastes by Cephalosporium eichhorniae. Appl Environ Microbiol 53:284–291
42. Jasti N, Khanal SK, Pometto AL III, van Leeuwen J(Hans) 2006 Fungal treatment of corn processing wastewater in an attached growth system. Water Pract Technol 1(3):115–122
43. Rasmussen ML, Khanal SK, Pometto AL III, van Leeuwen J(Hans) (2011) Water reclamation and value-added animal feed from corn ethanol stillage by fungal processing. Biomass Bioenergy (in press)
44. Kurakov AV, Popov AI (1996) Nitrifying activity and phytotoxicity of microscopic soil fungi. Eurasian Soil Sci 28:73–84
45. Jin B, Van Leeuwen J, Patel B, Yu Q (1999) Mycelial morphology and fungal protein production from starch processing wastewater in submerged cultures of aspergillus oryzae. Process Biochem 34:335–340
46. Moore-Landecker E (1990) Fundamentals of the fungi, 3rd edn. Prentice Hall, Upper Saddle River
47. Maheshwari R, Bharadwaj G, Bhat MK (2000) Thermophilic fungi: their physiology and enzymes. Microbiol Mol Biol Rev 64:461–488
48. Madigan MT, Martinko JM, Parker J (2000) Biology of microorganisms, 10th edn. Prentice Hall International Inc., London
49. Jin B, Yan XQ, Yu Q, Van Leeuwen J (2002) A comprehensive pilot plant system for fungal biomass protein production and wastewater reclamation. Adv Environ Res 6:179–189
50. Mishra BK, Arora A (2004) Optimization of a biological process for treating potato chips industry wastewater using a mixed culture of aspergillus foetidus and aspergillus niger. Bioresour Technol 94:9–12
51. Riscaldati E, Moresi M, Federici F, Petruccioli M (2000) Effect of pH and stirring rate on itaconate production by aspergillus terreus. J Biotechnol 83:219–230
52. Van Leeuwen J, Hu Z, Yi TW, Pometto AL III (2002) Use of micro-fungi for single cell protein production during food-processing wastewater treatment. In: Proceedings of the 75th water environmental federation technical exhibition and conference, Chicago, 30 Sept–2 Oct
53. Jin B, van Leeuwen J(Hans), Yu Q, Patel B (1999) Screening and selection of microfungi for microbial biomass protein production and water reclamation from starch processing wastewater. J Chem Technol Biotechnol 74:106–110
54. Metz B, Kossen NWF (1977) The growth of molds in the form of pellets-a literature review. Biotechnol Bioeng 19:781–799
55. Van Suijdam JC, Metz B (1981) Influence of engineering variables upon the morphology of filamentous molds. Biotechnol Bioeng 23:111–148
56. Tabak HH, Cooke WB (1968) The effects of gaseous environments on growth and metabolism of fungi. Bot Rev 34:126–252
57. U.S. Energy Information Administration via Farms.com News
58. USDA (2010) http://www.ers.usda.gov/Publications/FDS/2010/11Nov/ FDS10K01/
59. Renewable Fuels Association (RFA) (2009) Growing innovation: America's energy future starts at home. 2009 Ethanol Industry Outlook, Washington, D.C
60. Dunn L (2008) Personal communication through Lincolnway Energy, LLC
61. Schaefer SH, Sung S (2008) Retooling the ethanol industry: thermophilic anaerobic digestion of thin stillage for methane production and pollution prevention. Water Environ Res 80(2):101–108
62. Singh S, Fan M, Brown RC (2007) Ozone treatment of process water from a dry-mill ethanol plant. Bioresource Technol 99(6):1801–1805
63. RFA. Resource center: how ethanol is made. http://www.ethanolrfa.org/resource/made/. Accessed 10/01/09
64. USDA (2011) Market issues and prospects for U.S. distillers' grains: supply, use, and price relationships/FDS-10 k-01 economic research service
65. Sheep Industry News (2007) Ethanol boom gives rise to cheaper sheep rations, Becky Talley. http://sheepindustrynews.org 11(11) November. Accessed 23 March 2011

66. Dhiraj A, Vattem A, Shetty K (2002) Solid-state production of phenolic antioxidants from cranberry pomace by rhizopus oligosporus. Food Biotechnol 16(3):189–210
67. Gautam P, Sabu A, Pandey A, Szakacs G, Soccol CR (2002) Microbial production of extracellular phytase using polystyrene as inert solid support. Bioresour Technol 83(3):229–233
68. Nahas E (1988) Control of lipase production by rhizopus oligosporus under various growth conditions. J Gen Microbiol 134(1):227–233
69. Sutardi, Buckle KA (1988) Characterization of extra- and intracellular phytases from rhizopus oligosporus used in tempeh production. Int J Food Microbiol 6(1):67–79
70. Yanai K, Takaya N, Kojima N, Horiuchi H, Ohta A, Takagi M (1992) Purification of two chitinases from rhizopus oligosporus and isolation and sequencing of the encoding genes. J Bacteriol 174(22):7398–7406
71. Tan SC, Tan TK, Wong SM, Khor E (1996) The chitosan yield of zygomycetes at their optimum harvesting time. Carbohydr Polym 30:239–342
72. Rhodes RA, Hall HH, Anderson RF, Nelson GEN, Shekleton MC, Jackson RW (1961) Lysine, methionine, and tryptophan content of microorganisms III Molds. Appl Environ Microbiol 9(3):181–184
73. Jasti N, Khanal SK, Pometto AL III, van Leeuwen J(Hans) (2008) Converting corn wet milling effluent into high-value fungal biomass in an attached growth bioreactor. Biotechnol Bioeng 101(6):1223–1233
74. Jasti N, Rasmussen ML, Khanal SK, Pometto AL III, van Leeuwen J(Hans) (2009) Influence of selected operating parameters on fungal biomass production in corn ethanol wastewater. J Environ Eng 135:1106–1114
75. Jin B, Yu Q, van Leeuwen J(Hans) (2001) A bioprocessing mode for fungal biomass protein production and wastewater treatment using an external airlift bioreactor. J Chem Technol Biotechnol 76:1041–1048
76. Jin B, Yu Q, Yan XQ, van Leeuwen J (2001) Characterization and improvement of oxygen transfer in pilot plant external air-lift bioreactor for mycelial biomass production and wastewater treatment. World J Appl Microbiol Biotechnol 17:265–272
77. Jin B, Yu Q, van Leeuwen J, Hung YT (2009) Integrated biotechnological fungal biomass protein production and wastewater reclamation, environmental bioengineering. In: Wang LK, Tay JH, Tay STL, Hung YT (eds) Handbook of environmental engineering, vol 11. The Humana Press, Inc., Totowa, p 465
78. Rasmussen ML, Kambam Y, Khanal SK, Pometto AL III, van Leeuwen J(Hans) 2007 Thin stillage treatment from dry-grind ethanol plants with fungi. American Society of Agricultural and Biological Engineers (ASABE) annual international meeting, Minneapolis, USA
79. Sankaran S, Khanal SK, Pometto AL III, van Leeuwen J(Hans) (2008) Ozone as a selective disinfectant for nonaseptic fungal cultivation on corn-processing wastewater. Bioresour Technol 99(17):8265–8273
80. Jasti N, Khanal SK, Pometto AL III, Van Leeuwen J (2005) Attached growth fungal system for food-processing wastewater treatment and high value protein recovery. In: CD-ROM Proceedings of 78th annual conference & exposition (WEFTEC), Washington, D.C, 29 Oct–2 Nov 2005
81. Walker GM (1998) Yeast physiology and biotechnology. Wiley, 350pISBN:0-471-96446-8
82. Zhang ZY, Jin B, Kelly JM (2007) Production of lactic acid from renewable materials by rhizopus fungi. Biochem Eng J 35:251–263
83. Sparringa RA, Owens JD (1999) Causes of alkalinization in tempe solid substrate fermentation. Enzym Microb Technol 25:677–681
84. Cheeke PR (2005) Protein sources, 3rd edn. Upper Saddle River, USA, pp 91–144
85. Ozsoy HD, Kumbur H, Saha B, van Leeuwen J (2008) Use of Rhizopus oligosporus produced from food processing wastewater as a biosorbent for Cu(II) ions removal from the aqueous solutions. Bioresource Tech 99(11):4943–4948
86. Ozsoy HD, van Leeuwen J (2010) Pb(II) ions removal from aqueous solutions by rhizopus oligosporus produced from food processing wastewater, Chap 16. In: Canton KW (ed) Fluid

waste disposal. Environmental science, engineering and technology series, Nova Publishers, Hauppauge, pp 317–329, ISBN: 978-1-60741-915-0
87. Ozsoy HD, van Leeuwen J(H) (2011) Use of dried Rhizopus microsporus, produced from corn processing wastes, as adsorbent for metal removal from wastewater. In: Gopalakrishnan K, van Leeuwen J(H), Brown RC (eds) Sustainable bioenergy and bioproducts. Green energy and technology series VII. Springer, New York. (Chapter 3 (2012) , ISBN 978-1-4471-2323-1)
88. Shrestha P, Rasmussen ML, Nitayavardhana S, Khanal SK, van Leeuwen J (2010) Value-added processing of residues from biofuel industries, Chapter 17. In: Khanal SK, Zhang T, Surampalli R, Tyagi R, Lamsal B (eds) Biofuel and bioenergy from biowastes and residues. American society of civil engineers, p 522, ISBN-10: 0784410895
89. Khanal SK, Rasmussen ML, Shrestha P, Lamsal BP, van Leeuwen J, Visvanathan C, Liu H (2008) Bioenergy and biofuel from wastes/residues of emerging biofuel industries. Water Environ Res 80(10):1625–1647
90. Shrestha P (2006) Saccharification of corn fiber by phanerochaete chrysosporium in solid-state fermentation and subsequent fermentation of hydrolysate into ethanol. Iowa State University, M.S. thesis
91. Rasmussen ML, Shrestha P, Khanal SK, Pometto AL III, van Leeuwen J (2010) Sequential saccharification of corn fiber and ethanol production by the brown-rot fungus gloeophyllum trabeum. Bioresour Tech 101(10):3526–3533
92. Shrestha P, Khanal SK, Pometto AL, van Leeuwen J (2009) Corn fiber induced extracellular enzymes production by wood-rot and soft-rot fungi for subsequent fermentation of hydrolysate to ethanol. J Agric Food Chem 57(10):4156–4161
93. Vincent M, Pometto AL, van Leeuwen J(H) (2011) Simultaneous saccharification and fermentation of ground corn stover for the production of fuel ethanol using Phanerochaete chrysosporium, Geophyllum trabeum, Saccharomyces cerevisiae, and Escherichia col K011. J Microbiol Biotechnol 21(7):703–710
94. Vincent M, Pometto AL III, van Leeuwen J(H) (2011) Evanluation of potential fungal species for the in sity simultaneous saccharification and fermentation (SSF) of cellulosic material. Malaysian. J Microbiol 7(3):129–138
95. Shrestha P, Khanal SK, Pometto AL, Van Leeuwen J (2011) Study of cellulose degrading enzyme profiles during fungal saccharification of corn fiber. J Agric Food Chem (in press)
96. Shrestha P, Pometto AL III, Khanal SK, van Leeuwen J(Hans) (2012) Secondgeneration biofuel production from corn-ethanol industry residues. In: Gopalakrishnan K, van Leeuwen J(H), Brown RC (eds) Sustainable bioenergy and bioproducts. Springer, New York (Chapter 5)
97. Karki B, Lamsal BP, Grewell D, Pometto AL III, van Leeuwen J(Hans), Khanal SK, Jung S (2009) Functional properties of soy protein isolates produced from ultrasonicated defatted soy flakes. J American Oil Chem Society 86(10):1021–1028
98. Mitra D, Rasmussen ML, Chand P, Chintareddy VR, Yao L, Grewell D, Verkad JG, Wang T, van Leeuwen J(Hans) (2011) Bioresour Technol (in press)
99. Falanghe H, Smith AK, Rackis JJ (1964) Production of fungal mycelial proteinin submerged culture of soybean whey. Appl Environ Microbiol 12(4):330–334
100. Yokoi H, Aratake T, Nishio S, Hirose J (1998) Production of chitosan from mycelia of thefungus Gongronella butleri. Artificial Organs 22(10):837–848
101. Suntornsuk W, Pochanavanich P, Suntornsuk L (2002) Fungal chitosan production on food processing by-products. Process Biochem 37:727–729
102. O'Brien DJ, Heiland WK (1993) Attached growth biological reactor for the 1485growth and harvesting of filamentous fungi. US Patent No. 5246854
103. Farabee MJ (2001) http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookDiversity_4.html. Accessed 19 Nov 2005
104. Futcher T (2005) http://www.cs.cuc.edu/~tfutcher/Deuteromycota.html. Accessed 19 Nov 2005
105. El-Zalaki ME, Hamza MA (1979) Edible mushrooms as producers of amylases. Food Chem 4:203–211

106. NCIM (2005) Strains with special application: fungi. The national collection of industrial microorganisms (NCIM) catalogue, National chemical Laboratory. http://wdcm.nig.ac.jp/catalogue/ncim/document/Ncim_s_fungi.pdf, Pune, India. Accessed 20 June 2005
107. Allan GG, Fox JR, Kong N (1978) A critical evaluation of the potential sources of chitin and chitosan. In: Muzzarelli RAA, Pariser ER (ed) Proceedings of the first international conference on Chitin/Chitosan. MIT Sea grant report MITSG78-7, index no. 78-307-Dmb, Massachusetts Institute of Technology, Cambridge, pp 64–78
108. Knorr D, Klein J (1986) Production and conversion of chitosan with cultures of Mucor rouxii or phycomyces blakesleeanus. Biotechno Lett 8:691–694
109. Davoust N, Hansson G (1991) Identifying the conditions for development of beneficial mycelium morphology for chitosan-producing Absidia spp. in submersed cultures. Appl Microbiol Biotechnol 36:618–620
110. Andrade VS, Neto BB, Souza W, Takaki GMC (2000) A factorial design analysis of chitin production by cunninghamella elegans. Can J Microbiol 46:1042–1045
111. Manjunath P, Shenoy BC, Rao MR (1983) Review: fungal glucoamylases. J Appl Biochem 5:235–260
112. Stone PJ, Makoff AJ, Parish JH, Radford A (1993) Cloning and sequence analysis of the glucoamylase gene of Neurospora crassa. Curr Genet 24:205–211
113. Norouzian D, Akbarzadeh A, Scharer JM, Young MM (2006) Fungal glucoamylases. Biotechnol Adv 24:80–85
114. Rosenberg M, Kristofikova L (1995) Physiological restriction of the L-lactic acid production by rhizopus arrhizus. Acta Biotechnol 15:367–374
115. Mirdamadi S, Sadeghi H, Sharafi N, Falahpour M, Mohseni AF, Bakhtiari MR (2002) Comparison of lactic acid produced by fungal and bacterial strains. Iran Biomed J 6:69–75
116. Huang LP, Jin B, Lant P, Zhou J (2003) Biotechnological production of lactic acid integrated with potato wastewater treatment by rhizopus arrhizus. J Chem Technol Biotechnol 78:899–906
117. Data from National Research Center (1988) Nutrient requirements of swine, 10th edn. National Academies Press, Washington

Fungal Biosorption of Ni (II) Ions

H. Duygu Ozsoy and J. (Hans) van Leeuwen

Abstract Heavy metals are important pollutants released to the environment by human activities and natural processes. Industrial operations are the main source of dispersion of heavy metals into the environment. Nickel is one of these toxic heavy metals, which can affect human health and cause dermatitis, allergic sensitization, lung and nervous system damage. It is also a known carcinogen. According to the World Health Organization (WHO) guidelines for drinking water, the permissible level of Ni^{+2} is 5 ppm. Therefore, it is necessary to decrease the concentration of nickel to permissible limits before discharge. Biosorption using microbial biomass such as bacteria, fungi, yeast and algae, is regarded as a cost-effective biotechnology for the treatment of wastewaters containing heavy metals. This chapter includes experimental research on fungal biosorption of Ni (II) ions. Results indicate that it is possible to use dried *Rhizopus oligosporus* biomass to remove Ni (II) ions from their aqueous solutions. Food processing wastewater can be used as a substrate for cultivating the fungal biomass to reduce operational costs of biosorption process. The biosorption process was carried out in a batch process and the effects of contact time (1–48 h), initial pH (2.0–7.0), initial metal ion concentration

H. Duygu Ozsoy (✉)
Engineering Faculty, Department of Environmantal Engineering,
Mersin University, 33363 Mersin, Turkey
e-mail: ozsoyhd@mersin.edu.tr

J. (Hans) van Leeuwen
Department of Civil, Construction and Environmental Engineering,
Department of Agricultural and Biosystems Engineering,
Department of Food Science and Human Nutrition,
Iowa State University, Ames, IA 50011, USA

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*,
Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_3,

(20–100 mg/L) and biosorbent dosage (0.5–5 g/L) on the biosorption were investigated. Experimental results showed that the biosorption onto dried fungal biomass could be well described by the Langmuir equations and the Langmuir monolayer capacity had a mean value of 116 mg/g. Pseudo-second-order reaction model provided the best description of the data with a correlation coefficient 0.99 for different initial metal concentrations. This result indicates that chemical sorption might be a basic mechanism in this system.

1 Biosorption of Nickel

Heavy metals belong to an important group of pollutant released to the environment by human activities and natural pathways. Industrial operations are the main source of dispersion of heavy metals into receiving waters. Although living organisms require varying amounts of heavy metals such as copper, zinc, iron, and manganese excessive levels cause toxic and inhibitory effects. Therefore, metal contaminated effluents should be treated before discharge [46].

Nickel (II) ion is a heavy metal frequently encountered in raw wastewater streams from industries such as electroplating, battery production, non-ferrous metal, mineral processing, coal-fired power plants and copper sulfate manufacture. [35]. This heavy metal can affect human health and cause dermatitis, allergic sensitization, lung and nervous system damage. It is also a known carcinogen. In drinking water, nickel may cause health problems if consumed in amounts greater than the health standard (MCLG: 0.1 mg/L) set by the United States Environmental Protection Agency (EPA). According to the World Health Organization (WHO) guidelines for drinking water, the permissible level of Ni^{+2} is 5 ppm. Therefore, it is necessary to decrease the concentration of nickel to permissible limits before discharge to waters that may ultimately serve as a drinking water source [28].

The traditional methods for the treatment of metal-contaminated wastewater include chemical precipitation, chemical oxidation or reduction, electrochemical treatment, reverse osmosis, solvent extraction, ion exchange and evaporation. However, these techniques have several demerits such as high chemical cost, low removal efficiency, low selectivity, high energy requirements and generation of secondary toxic slurries [1, 7, 8]. Biosorption using microbial biomass such as bacteria, fungi, yeast and algae, is regarded as a costeffective biotechnology for the treatment of wastewaters containing heavy metals. Biosorbents are not only cost effective; but also provide an opportunity for the recovery and recycling of waste materials [13, 16, 23, 27, 33]. Another advantage is that the biomaterials used are cheap enough to be used on a disposable basis so that no regeneration is required. Table 1 shows different biosorbents and their biosorption capacities for Ni (II) ions.

Table 1 Different biosorbents and their biosorption capacities for Ni (II) ions

Biosorbent	q_{max} (mg/g)	Reference
Rice hull	5.75	[41]
Thuja orientalis biomass	12.42	[28]
Olive stone waste	1.87	[11]
Raw rice bran	0.15	[30]
Dried *Chlorella vulgaris*	58.40	[3]
Reed biomass	7.92	[40]
Mucor rouxil	5.24	[47]
Rhizopus arrhizus	18.00	[12]
Aspergillus niger	3.9	[25]
Rhizopus nigricans	5.0	[21]
Enteromorpha prolifera	36.8	[31]
Saccharomyces rimosus	1.5	[4]
Sugar beet pulp (acid extracted)	10.74	[37]
Chlorella vulgaris	63.25	[2]
Cyanobacteria *Nostoc sp.*	9.0	[9]
Candida cells (adapted)	30.8	[10]
Oedogonium hatei (acid treated)	44.2	[15]
Oedogonium hatei (untreated)	40.9	[15]
Deactivated protonated yeast	9.0	[35]
Gracilaria caudate	45.0	[14]
Sargassum muticum	70.0	
Punica granatum peel waste	52.0	[5]
Moringa oleifera bark	30.4	[26]

The cellwalls of biomass play an important role in sorption and therefore also in the removal of Ni (II) ions. Fungal biomass is a complex material containing protein, lipids and polysaccharides, mainly chitin, as major constituents. The polar functional groups of chitin, which include amino, hydroxyl and carboxyl groups as chemical bonding agents can be responsible for chemisorption of nickel.

Fungal biosorption of Ni (II) ions, like other metals, largely depends on water quality determinants such as pH, metal ion and biomass concentration, physical or chemical pretreatment of biomass, presence of various ligands in solution and temperature. Fungal biosorption performs well in comparison to sorption on commercial ion-exchange resins, activated carbon and metal oxides [24].

Cultivation of microorganisms requires a bioreactor and nutrients such as carbon, nitrogen and trace elements [29, 49]. Therefore, cultivation cost is the most important factor to produce these biosorbents [38, 39, 43, 44]. Experimental results indicated that food-processing wastewater can be used as a substrate for cultivating fungal biomass to reduce operational costs of biosorption process [34]. *R. oligosporus* is a fungus of the family Mucoraceae that is easily grown in food-processing wastewater. Using the wastewaters would be particularly attractive and cost-effective because there are many food-processing plants in Turkey, USA and

many other countries that could provide suitable industrial wastewaters for cultivating fungal biomass, such as *R. oligosporus*.

This chapter includes research results on fungal biosorption of Ni (II) ions. The goal of the research was to investigate the efficacy of dried *R. oligosporus* fungal biomass as a biosorbent for removal of Ni (II) ions from aqueous solution. The effects of contact time, initial pH, initial metal ion concentration and temperature on biosorption efficiency and mechanism of the biosorption were evaluated on laboratory scale.

2 Experimental

This research comprised two phases. *R. oligosporus* were grown on food-processing wastewater and the harvested and dried fungi was used for biosorption of Ni (II) ions.

2.1 Cultivation of R. oligosporus

Rhizopus oligosporus was obtained from American Type Culture Collection (Rockville, MD). The culture was rehydrated and revived in yeast-malt (YM) nutrient broth at 24°C. The revived culture was transferred on to numerous potato dextrose agar (PDA) plates and incubated at room temperature (24°C) for 7 days. Then fungal sporangiospores were harvested from the surface of PDA plates into sterile distilled water containing 0.85% (w/v) saline solution (NaCl) and 0.5% (v/v) of Tween 80. The harvested cultures were diluted further to achieve a spore count of 10^6–10^7 spores/ml, determined by hemocytometer counts. Glycerin (20%; v/v) was added to the spore suspension as a cryoprotectant for ultra-low frozen storage at −75°C in 2 ml cryo-vials for future use as a bioreactor inoculum.

The inocula were used to seed laboratory-scale continuous attached growth tank reactors using corn-processing wastewater as organic substrate. The wastewater was supplied from the ADM wet-corn milling facility in Cedar Rapids, IA, US. The reactors were operated at a hydraulic retention time (HRT) of 8 h and solids retention time (SRT) of 2 days. These HRT and SRT values were found to be optimal for the maximum growth of the *Rhizopus oligosporus* [22]. The microfungi were growing in the form of attached mycelia and harvested daily from the bioreactor by natural sloughing off the attachment surface and subsequent gravity settling. The mycelia were washed with deionised water and dried at 65°C for 24 h. The dried fungal pellets were ground and sieved (0.5 mm < diameter).

2.2 Chemical Solutions

A 1 g/L stock solution of nickel was prepared with single reagent grade metal solution (Claritas, Fisher Chemicals) in deionized water. The metal solution was diluted to appropriate concentrations as needed and stored at 4°C until further use. HNO_3 and NaOH were obtained from Fisher Chemicals and used for pH value adjustment.

2.3 Batch Biosorption Experiments of Ni (II) Ions

The effects of contact time, initial pH, initial metal ion concentration and temperature on biosorption efficiency were examined through a series of shaker flask tests. After determining the optimum conditions, a series of biosorption tests were conducted to determine isotherms for Ni (II) ions.

All sorption tests were conducted using single reagent grade metal to minimize the variability of metal concentrations and to avoid competitive biosorption of mixed metals on biosorbent. A total of 100 ml of metal solution were added to each flask containing 0.1 g (dry weight) of *R. oligosporus*. The flasks were placed on an orbital shaker table running at 150 rpm at 30 ± 1°C (except the temperature experiments) until equilibrium was reached. The residual concentration of Ni (II) ions in the aqueous phase (obtained by centrifugation, 1000 g-10 min) was determined using Inductively Coupled Plasma–Mass Spectrometer (ICP–MS). All tests were conducted in triplicate.

The concentrations of the Ni (II) ions the in aqueous phase were used to determine the biosorption capacity of *R. oligosporus*. Equilibrium sorption isotherms were determined by mass balance. The solid phase Ni (II) concen-tration at (pseudo) equilibrium, q_{eq} (mg/g) was calculated as follows:

$$q_{eq} = \frac{[(C_o - C_{eq})V]}{x} \tag{1}$$

where C_o and C_{eq} are the initial and equilibrium concentrations of Ni (II) ions (mg/L), V volume of solution and x the weight of sorbent (g).

2.4 ICP–MS Analysis

A Hewlett Packard 4500 Series ICP–MS was used with external calibration. The instrument was calibrated before each measurement. Operating parameters are summarized in Table 2.

Table 2 ICP-MS operating conditions

R_f power	1200 W
R_f matching	2 V
Sample depth	7.8 mm
Plasma gas flow	16 L/min
Auxiliary gas flow	1.4 L/min
Carrier gas flow	1.0 L/min
Acquisition time	22.83 s
Resolution	300

2.5 Equilibrium Isotherms and Kinetics of Biosorption

The Langmuir isotherm was used to describe observed sorption phenomena. The Langmuir isotherm applies to biosorption on completely homogenous surface with negligible interaction between adsorbed molecules [17, 48]. The linearized form of the equation can be written as;

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{bq_{\max}} + \frac{C_{eq}}{q_{\max}} \tag{2}$$

where C_{eq} is the equilibrium concentration of Ni (II) ions, q_{eq} is the amount of biosorption at equilibrium, q_{max} is the monolayer capacity and b is an equilibrium constant of Langmuir. The Freundlich isotherm (empirical model adsorption in aqueous systems) was also tested with our experimental data. The linearized form of the equation can be written as:

$$\ln q_{eq} = \ln K_f + \frac{1}{n} \ln C_{eq} \tag{3}$$

where K_f is the measure of sorption capacity and 1/n is sorption intensity.

Pseudo-first-order and pseudo-second-order kinetic models were applied to analyze the sorption kinetics of Ni (II) ions. A simple pseudo-first-order equation due to Lagergren was used by different researchers [6, 19]:

$$\log\left(q_{eq} - q_t\right) = \log q_{eq} - \frac{k_{ad}}{2.303t} \tag{4}$$

where q_e and q_t are the amount of adsorption at equilibrium and at time t, respectively, and k_{ad} is the rate constant of the pseudo-first-order adsorption process.

A plot of log $(q_{eq}-q_t)$ vs. t would provide a straight line for first-order adsorption kinetics, allowing computation of the adsorption rate constant, k_{ad}. Ho's second-order rate equation which has been called pseudo-second-order kinetic expression has also been applied widely [18, 20]. The linear form of the kinetic rate equations can be written as follows:

$$\frac{t}{q_t} = \frac{1}{kq_{eq}^2} + \frac{1}{q_{eq}} t \tag{5}$$

where k is the rate constant of sorption (L/mg min), q_{eq} is the amount of metal ion sorbed at equilibrium (mg/g) and q_t is the amount of metal ion sorbet at time t (mg/g). The constants can be determined experimentally by plotting of t/q_t against t.

2.6 SEM and EDX Analysis

Scanning Electron Microscopy (SEM) can characterize metal accumulation on the biosorbent surface. For this reason, the surface structure of the dried *R. oligosporus* biomass before and after Ni (II) ions adsorption was also analyzed by scanning electron microscopy coupled with Energy Dispersive X-ray (EDX) analysis on a Hitachi S-2460 N instrument.

3 Experimental Results

3.1 The effect of contact time

Figure 1 shows the effect of contact time on biosorption of Ni (II) ions (100 mg/L) onto the dried *R. oligosporus* biomass. Ni (II) biosorption capacity increased during the first 6 h and the leveled off toward the equilibrium biosorption capacity. For this reason contact times of 6 h were used for further experiments.

3.2 The effect of the initial pH

Results of the experiments using 100 mg/L Ni (II) solutions and 1 g/L biosorbent showed that efficiencies of biosorption were increased with increasing pH from 2.0 to 6.0 (Fig. 2). At low pH values, the surface of biosorbent would also be surrounded by hydronium ions, which decrease the nickel interaction with binding sites of the dry fungi by greater repulsive forces. As the pH increased, the overall surface on the dry fungi became negatively charged and biosorption increased [32]. At pH values above pH 5.0, turbidity was observed in the medium. This can be explained by precipitation of nickel ions from solution. Similarly, some researchers reported that pH 5.0 was the optimum pH value for removal of Ni (II) ions from aqueous solutions [11, 30, 42, 45].

3.3 The effect of the initial metal concentrations

Biosorption capacities of the dried *R. oligosporus* biomass for the Ni (II) ions were studied as a function of the initial Ni (II) ions concentration between 20 and 100 mg/L in the biosorption medium (Fig. 3).

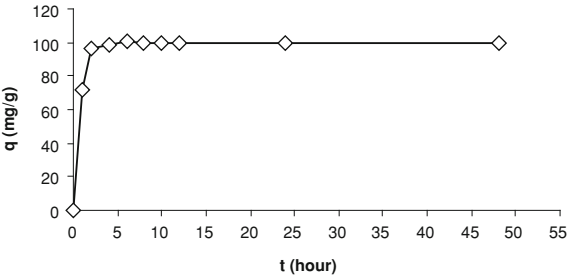

Fig. 1 The effect of contact time on biosorption of Ni (II) ions (100 mg/L) to the dried fungi (biosorbent dosage 1 g/L; pH: 5.0; 30°C)

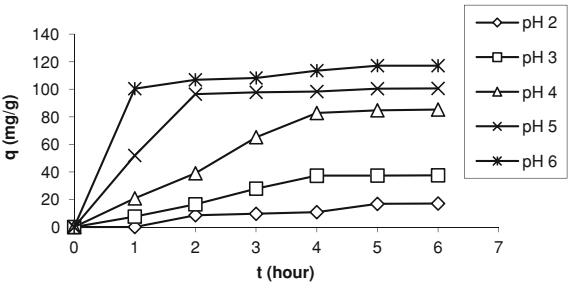

Fig. 2 The effect of the initial pH on biosorption of Ni (II) ions (100 mg/L) to the dry fungi (biosorbent dosage 1 g/L; contact time 6 h; 30°C)

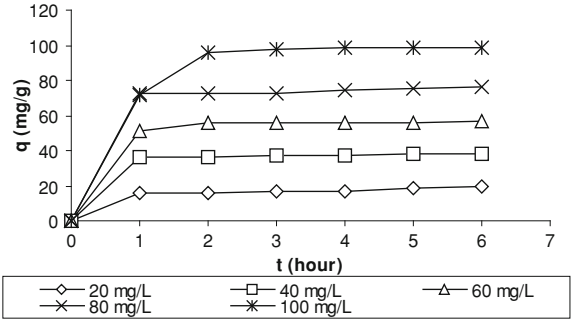

Fig. 3 The effect of initial metal concentrations on biosorption of Ni (II) ions on the fungi (biosorbent dosage 1 g/L; contact time 6 h; pH: 5.0)

Akar et al. [1] indicated that biosorption of Ni (II) ions onto silica-gel-immobilized waste biomass increased with increasing initial metal concentration. The biosorption of Ni (II) onto immobilized biomass was studied at different initial Ni (II) ion concentrations ranging from 75 to 500 mg/L by the researchers and they reported that the maximum equilibrium capacity (90 mg/g) was found at the highest initial concentration of 500 mg/L.

3.4 The effect of biosorbent dosage

Nickel (II) biosorption on dried *R. oligosporus* biomass was studied at various biosorbent dosages ranging from 0.5 to 5 g/L. Experimental results indicated that the efficiency of biosorption decreased with increasing biosorbent dosage (Fig. 4).

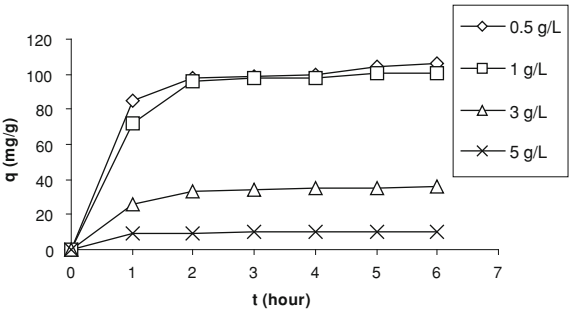

Fig. 4 The effects of biosorbent dosage on biosorption of Ni (II) on the dry fungi (contact time 6 h; pH: 5.0; 30°C)

Aggregation of biosorbent particles could prevent the sorption process in the presence of higher biomass amounts. In addition, the biosorption sites remain unsaturated during the sorption process in these conditions. As a result, more economical removal of a given amount of metal ions can be obtained using consecutive small batches of biosorbent rather than a single batch [30].

3.5 Isotherms and Kinetics

In order to investigate the sorption isotherm for dried *R. oligosporus* biomass, two equilibrium models were employed: The Langmuir and Freundlich isotherm equations. The correlation coefficient of the Freundlich isotherm (R^2) was 0.8511 (Fig. 5). The Langmuir model was the best-fit isotherm for biosorption of Ni (II) to the dried *R. oligosporus* biomass. Langmuir isotherm model parameters, q_{max} and b, were estimated from the intercept and slope of C_{eq}/q_{eq} vs. C_{eq}, according to Eq. (2) and obtained as 116.28 (mg/g) and 0.327 (L/g), respectively. The correlation coefficient of Langmuir isotherm (R^2) was 0.9904 (Fig. 6).

Pahlavanzadeh et al. [36] also indicated that the correlation coefficients for the Freundlich adsorption isotherm model for removal of Ni (II) ions onto brown algae (*Cystoseria indica, Nizmuddinia zanardini, Sargassum glaucescens* and *Padina australis*) were found to be lower than Langmuir adsorption isotherm model coefficients (>0.995).

A comparison of the maximum capacity, qmax, of the dried R. oligosporus biomass (116.28 mg/g) with those of some other adsorbents reported in the literature (Table 1) shows that the dried *R. oligosporus* biomass has good biosorption capacity. The biosorption capacity differences of metal uptake are due to the properties of each biosorbent such as structure, functional groups and surface area.

Kinetic studies were carried out for biosorption of Ni (II) as a function of contact time at various initial Ni (II) concentrations ranging from 20 to 100 mg/L. Experimental results indicated that the pseudo-second-order reaction model provided the best description of the data with a correlation coefficient 0.99 for

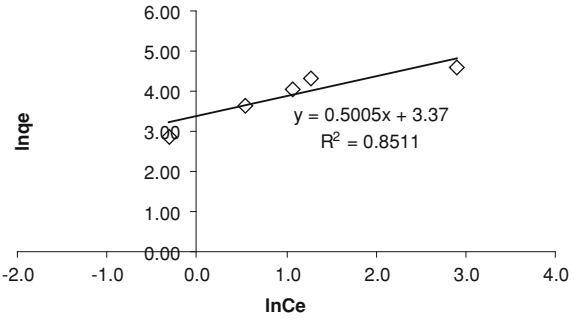

Fig. 5 The Freundlich adsorption isotherms for Ni (II) biosorption by dry fungi (Biosorbent dosage, 1 g/L; pH 5; initial metal concentration 20–100 mg/L; 30°C)

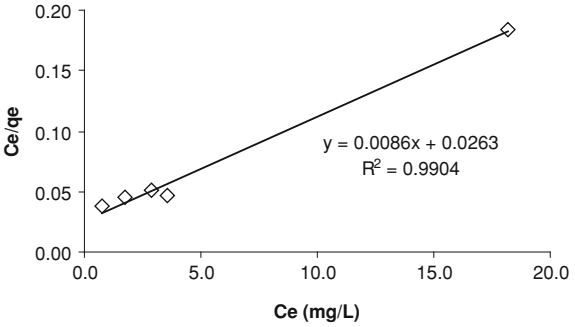

Fig. 6 The Langmuir adsorption isotherm for Ni (II) biosorption on dry fungi (biosorbent dosage 1 g/L; pH 5.0; initial metal concentration 20–100 mg/L; 30°C)

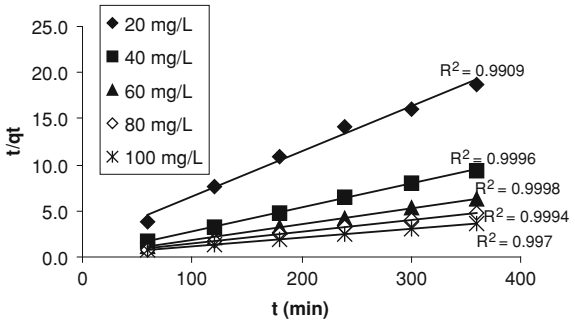

Fig. 7 The plots of pseudo-second-order kinetics with respect to different initial Ni (II) ion concentrations (Biosorbent dosage 1 g/L; pH 5.0; 30°C)

different initial metal concentrations (Fig. 7). Reaction rate constants for pseudo-second-order equations are shown at Table 3. The results indicated that the biosorption system studied follows a pseudo-second-order kinetic model at all time intervals. Similarly, Padmavathy [35] reported kinetics of biosorption of nickel (II) ion removal by baker's yeast to follow a pseudo-second-order reaction.

Table 3 Pseudo-second-order reaction rate regression results

Initial metal conc.(mg/L)	q_{exp} (mg/g)	q_{cal} (mg/g)	Correlation coeff. (R^2)
20	19.27	20.45	0.9909
40	38.29	38.61	0.9996
60	57.11	57.80	0.9998
80	76.43	76.92	0.9940
100	99.00	105.26	0.9970

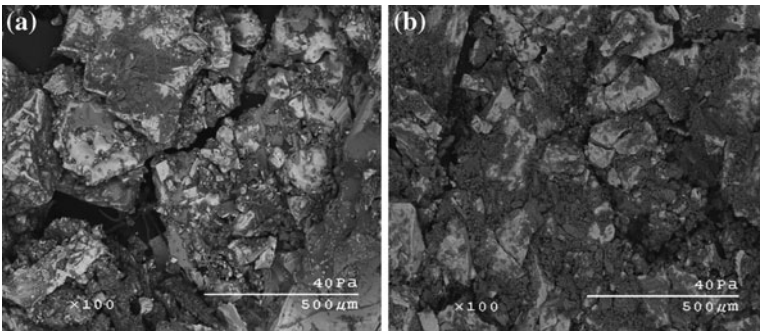

Fig. 8 SEM micrograph of dried *R. oligosporus* biomass before (**a**) and after (**b**) the Ni (II) biosorption. (Bar 500 μm)

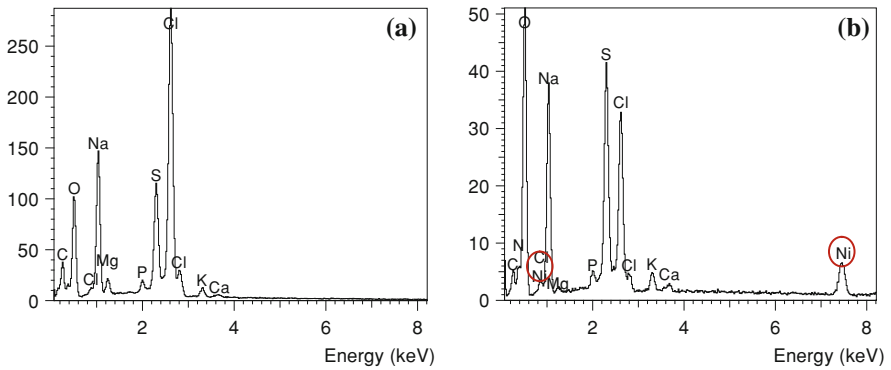

Fig. 9 EDX spectra of dried *R. oligosporus* biomass before (**a**) and after (**b**) the Ni (II) biosorption

3.6 Results of SEM and EDX Analysis

Scanning Electron Microscopy, SEM, of dried *R. oligosporus* biomass before and after biosorption is shown in Fig. 8a, b.

The nickel on the biosorbent surface was then characterized by Energy Dispersive X-ray spectrometry. The EDX analysis shown in Fig. 9 reveals nickel signals on the surface of biomass before (a) and after (b) metal biosorption.

4 Conclusion

Biosorption of fungal biomass is a cost-effective biotechnology for the treatment of nickel-contaminated wastewaters. The potential use of dried *R. oligosporus* biomass as a biosorbent for nickel is possible. On the other hand, it is possible to use food-processing wastewater as a substrate for cultivating the fungal biomass and reduce operational costs of biosorption process. Experimental results indicated that the maximum Ni (II) biosorption capacity was 116 mg Ni (II)/g of dried *R. oligosporus* biomass (biosorbent dose of 1 g/L, contact time:6 h, initial Ni (II) concentration:100 mg/L, pH 5.0). The pseudo-second-order reaction model provided the best description of the data with a correlation coefficient 0.99 for different initial metal concentrations. The fit of the experimental data to this model suggests that the process controlling the rate may be a chemical sorption.

Dried *R. oligosporus* biomass has the potential to be used as an effective and economic biosorbent material for the removal of Ni (II) ions from wastewaters.

References

1. Akar T, Kaynak Z, Ulusoy S, Yuvaci D, Ozsari G, Akar ST (2009) Enhanced biosorption of nickel (II) ions by silica-gel-immobilized waste biomass: biosorption characteristics in batch and dynamic flow mode. J Hazard Mater 163:1134–1141
2. Aksu Z (2002) Determination of the equilibrium, kinetic and thermodynamic parameters of the batch biosorption of nickel(II) ions onto *Chlorella vulgaris*. Process Biochem 38:89–99
3. Aksu Z, Donmez G (2006) Binary biosorption of cadmium(II) and nickel(II) onto dried Chlorella vulgaris: co-ion effect on mono-component isotherm parameters. Process Biochem 41:860–868
4. Bakkaloglu I, Butter TJ, Evison LM, Holland FS, Hancock IC (1998) Screening of various types of biomass for removal and recovery of heavy metals (Zn, Cu, Ni) by biosorption, sedimentation and desorption. Water Sci Technol 38:269–277
5. Bhatnagar A, Minocha AK (2010) Biosorption optimization of nickel removal from water using *Punica granatum* peel waste. Colloids Surf B: Biointerfaces 76:544–548
6. Bhattacharyya KG, Sharma A (2005) Kinetics and thermodynamics of Methylene Blue adsorption on neem (*Azadirachta indica*) leaf powder. Dye Pigment 65:51–59
7. Chang WC, Hsu GS, Chiang SM, Su MC (2006) Heavy metal removal from aqueous solution by wasted biomass from a combined AS-biofilm process. Bioresour Technol 97:1503–1508
8. Chen JZ, Tao XC, Xu J, Zhang T, Liu ZL (2005) Biosorption of lead, cadmium and mercury by immobilized *Microcystis aeuroginosa* in a column. Process Biochem 40:3675–3679
9. Corder SL, Reeves M (1994) Biosorption of nickel in complex aqueous waste streams by cyanobacteria. Appl Biochem Biotechnol 45–46:847–859

10. Donmez G, Aksu Z (2001) Bioaccumulation of copper(II) and nickel(II) by the nonadapted and adapted growing *Candida* sp. Water Res 35:1425–1434
11. Fiol N, Villaescusa I, Martinez M, Miralles N, Poch J, Serarols J (2006) Sorption of Pb(II), Ni(II), Cu(II) and Cd(II) from aqueous solution by olive stone waste. Sep Purif Technol 50:132–140
12. Fourest E, Roux JC (1992) Heavy metal biosorption by fungal mycelial by-products: mechanism and influence of pH. Appl Microbiol Biotechnol 37:399–403
13. Garg VK, Gupta R, Kumar R, Gupta RK (2004) Adsorption of chromium from aqueous solution on treated sawdust. Bioresour Technol 92:79–81
14. Gonzalez Bermudez Y, Rodriguez Rico IL, Gutierrez Bermudez O, Guibal E (2011) Nickel biosorption using *Gracilaria caudate* and *Sargassum muticum*. Chem Eng J 166:122–131
15. Gupta VK, Rastogi A, Nayak A (2010) Biosorption of nickel onto treated alga (*Oedogonium hatei*): Application of isotherm and kinetic models. J Colloid Interface Sci 342:533–539
16. Hanzlik J, Jehlicka J, Sebek O, Weishauptova Z, Machovic V (2004) Multi-component adsorption of Ag(I), Cd(II) and Cu(II) by natural carbonaceous materials. Water Res 38:2178–2184
17. Ho YS (2004) Pseudo-isotherms using a second order kinetic expression constant. Adsorption 10:151–224
18. Ho YS (2005) Effect of pH on lead removal from water using tree fern as the sorbent. Bioresour Technol 96:1292–1296
19. Ho YS, McKay G (1998) Sorption of dye from aqueous solution by peat. Chem Eng J 70:115–124
20. Ho YS, McKay G (2000) The kinetics of sorption of divalent metal ions onto sphagnum moss peat. Water Res 34(3):735–742
21. Holan B, Volesky B (1994) Biosorption of lead and nickel by biomass of marine algae. Biotechnol Bioeng 43:1001–1009
22. Jasti N, Khanal SK, Pometto AL III, van Leeuwen J (2006) Fungal treatment of corn processing wastewater in an attached growth system. Water Sci Prac 1(3):1–8
23. Kadirvelu K, Kavipriya M, Karthika C, Radhika M, Vennilamani N, Pattabhi S (2003) Utilization of various agricultural wastes for activated carbon preparation and application for the removal of dyes and metal ions from aqueous solution. Bioresour Technol 87:129–132
24. Kapoor A, Viraraghavan T (1995) Fungal biosorption—an alternative treatment option for heavy metal bearing wastewaters: a review. Bioresour Technol 53(3):195–206
25. Kapoor A, Viraraghavan T, Cullimore P (1999) Removal of heavy metals using the fungus *Aspergillus niger*. Biores Technol 70:95–104
26. Kumar Reddy DH, Ramana DKV, Seshaiah K, Reddy AVR (2011) Biosorption of Ni(II) from aqueous phase by *Moringa oleifera* bark, a low cost biosorbent. Desalination 268:150–157
27. Ma W, Tobin JM (2004) Determination and modelling of effects of pH on peat biosorption of chromium, copper and cadmium. Biochem Eng J 18:33–40
28. Malkoc E (2006) Ni(II) removal from aqueous solutions using cone biomass of *Thuja orientalis*, J. Hazard Mater 137:899–908
29. Mazmanci MA, Unyayar A (2005) Decolorisation of reactive black 5 by *Funalia trogii* immobilized on *Luffa cylindirica* sponge. Process Biochem 40:337–342
30. Oliveria EA, Montanher SF, Andrade AD, Nobrega JA, Rollemberg MC (2005) Equilibrium studies for the sorption of chromium and nickel from aqueous solutions using raw rice bran. Process Biochem 40:3485–3490
31. Ozer A, Gurbuz G, Calimli A, Korbahti BK (2008) Investigation of nickel(II) biosorption on *Enteromorpha prolifera*: Optimization using response surface analysis. J Hazard Mater 152(2):778–788
32. Ozsoy HD, Kumbur H (2006) Adsorption of Cu(II) ions on cotton boll. J Hazard Mater B136:911–916
33. Ozsoy HD, Kumbur H, Ozer Z (2007) Adsorption of copper (II) ions to peanut hulls and Pinus brutia sawdust. Int J Environ Pollut 31(1–2):125–134

34. Ozsoy HD, Kumbur H, Saha B, van Leeuwen J (2008) Use of *Rhizopus oligosporus* produced from food processing wastewater as a biosorbent for Cu(II) ions removal from the aqueous solutions. Bioresource Tech 99(11):4943–4948
35. Padmavathy V (2008) Biosorption of nickel (II) ions by baker's yeast: Kinetic, thermodynamic and desorption studies. Bioresource Technol 99:3100–3109
36. Pahlavanzadeh H, Keshtkar AR, Safdari J, Abadi Z (2010) Biosorption of nickel (II) from aqueous solution by brown algae: equilibrium, dynamic and thermodynamic studies. J Hazard Mater 175:304–310
37. Reddad Z, Gerente C, Andres Y, Ralet MC, Thibault JF, Cloirec PL (2002) Ni(II) and Cu(II) binding properties of native and modified sugar beet pulp. Carbohydr Polym 49:23–31
38. Rasmussen ML, Shrestha P, Khanal SK, Pometto AL III, van Leeuwen J (2010) Sequential saccharification of corn fiber and ethanol production by the brown-rot fungus Gloeophyllum trabeum. Bioresource Tech 101(10):3526–3533
39. Sankaran S, Khanal SK, Pometto AL III, (Hans) van Leeuwen J (2008) Ozone as a selective disinfectant for nonaseptic fungal cultivation on corn-processing wastewater. Bioresour Technol 99(17):8265–8273
40. Southichak B, Nakano K, Nomura M, Chiba N, Nishimura O (2006) *Pragmites australis* : a novel biosorbent for the removal of heavy metals from aqueous solution. Water Res 40:2295–2302
41. Suemitsu R, Uenishi R, Akashi I, Nakan M (1986) The use of dyestuff-treated rice hulls for removal of heavy metals from wastewater. J Appl Polym Sci 31:74–83
42. Thevannan A, Mungroo R, Niu CH (2010) Biosorption of nickel with barley straw. Biores Tech 101:1776–1780
43. Van Leeuwen J (Hans), Hu Z, Yi TW, Pometto AL III, Jin B (2003) Kinetic model for selective cultivation of microfungi in a microscreen process for food processing wastewater treatment and biomass production. Acta Biotechnol 23:289–300
44. Van Leeuwen J (Hans) et al. (2011) Fungal treatment of crop processing wastewaters with value-added coproducts. Chapter in "sustainable bioenergy and bioproducts". In: Gopalakrishnan K, van Leeuwen J (H), Brown RC (eds) Springer (in press)
45. Vinod VTP, Sashidhar RB, Sreedhar B (2010) Biosorption of nickel and total chromium from aqueous solution by gum kondagogu (Coclospermum gossypium): A carbohydrate biopolymer. J Hazard Mater 178:851–860
46. Wang J, Chen C (2006) Biosorption of heavy metals by Saccharomyces cerevisiae: a review. Biotechnol Adv 24:427–451
47. Yan G, Viraraghavan T (2003) Heavy-metal removal from aqueous solution by fungus Mucor rouxii. Water Res 37:4486–4496
48. Yu B, Zhang Y, Shukla A, Shukla SS, Dorris KL (2001) The removal of heavy metals from aqueous solutions by sawdust adsorption-removal of lead and comparison of its adsorption with copper. J Hazard Mater B84:83–94
49. Yurekli F, Unyayar A, Porgali ZB, Mazmanci MA (2004) Effects of cadmium exposure on phytochelatin and the synthesis of abscisic acid in *Funalia trogii*. Eng Life Sci 4:378–380

Biomass Pretreatment for Biofuel Production

Devin Takara and Samir Kumar Khanal

Abstract Volatile oil prices and an increasing world demand for energy exemplifies the critical need for renewable sources of energy, particularly in the transportation sector, which can displace a significant fraction of fossil fuels imports. Following a shift in focus from first generation starch- and sugar-based feedstocks to second generation lignocellulosic biomass, a multitude of biomass pretreatment techniques and processes has been developed to facilitate the bioconversion of plant structural carbohydrates to fermentable sugars (for biofuels production). The state-of-the-art advancements to date in the pretreatment of second generation feedstocks have been considered, with an emphasis on the maximum yields and barriers of each technology. A brief discussion on the biorefinery concept, its applications, and the potential future of biofuels has also been included.

1 Introduction

In the wake of growing environmental and governmental pressures, safe, clean and renewable energy has become the primary focus of nations around the globe. The world's current energy consumption is projected to increase by 1.4% annually from 2007 to 2035, accounting for a 49% growth in total demand [1, 2]. Much of this demand will be met by fossil-derived resources originating from unstable regions of the world.

The transportation sector in particular, has received a considerable amount of attention based on its potential to displace a significant fraction of nonrenewable

D. Takara · S. K. Khanal (✉)
University of Hawaii at Manoa, Honolulu, HI, USA
e-mail: khanal@hawaii.edu.

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*, Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_4,

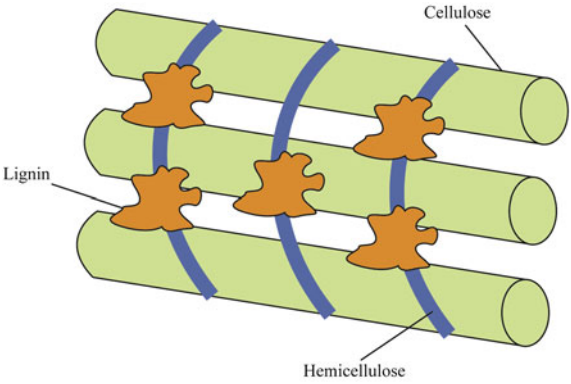

Fig. 1 Schematic illustration of lignin, cellulose, and hemicellulose interactions

resources, and reduce the volatility of fuel supply. In 2007, about 27% of the total energy consumed was used for transportation, and this value is expected to increase by 1.3% per year. Finding renewable resources is imperative for both sustainability and national security, and biofuels are key in reducing the strain on depleting petroleum reserves.

The first attempts to produce renewable fuel focused primarily on sugar and starchy crops as feedstocks. One of the challenges of using these crops (e.g. sugarcane, corn and soybean) however, is the resultant increase in prices for the food/feed industries and competition of land and resources [3]. These so-called first generation feedstocks can be easily converted to biofuel, but food-versus-fuel debates have placed an strong emphasis on second generation feedstocks, such as lignocellulosic biomass in the US and European Union [4]. In contrast to first generation feedstocks, second generation feedstocks minimize competition with the food industry and encompass a larger variety of biorenewable resources (e.g. agricultural and forest residues, and energy crops). These types of biomass, however, are intrinsically recalcitrant based on its biochemical composition.

Lignocellulosic biomass consists primarily of lignin (10–25%), cellulose (35–50%), and hemicellulose (20–35%) [5], as well as other components like protein and oils in lesser quantities (See Fig. 1). The building blocks of second generation feedstock structures consist of cellulose: a polysaccharide of glucose monomers joined by $\beta 1 \rightarrow 4$ glycosidic bond that arranges itself into linear strands stabilized by hydrogen bonding. These strands are reinforced by hemicellulose, a heteropolymer consisting of a mixture of five and six carbon sugars. Lignin, a polymer of p-coumaryl, coniferyl and sinapyl alcohols synthesized from sugars [6], binds to the hemicellulose, adding further reinforcement to the overall structure (Fig. 1). The ratio of the three lignin alcohols varies significantly from crop to crop, but the removal of lignin to increase the accessibility of enzymes to (hemi)cellulosic sugars remains an obstacle for all second generation feedstocks in biochemical conversions to biofuel. Although naturally occurring species like white-rot fungi have demonstrated the ability to degrade lignin [7], this process is too slow to be of value for commercial applications.

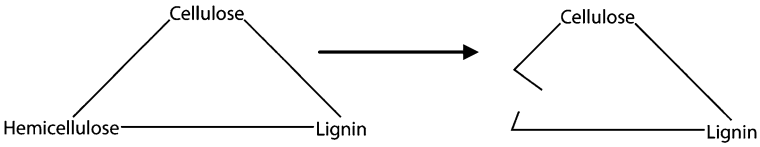

Fig. 2 Simplified schematic of plant composition after acid pretreatment

A plethora of pretreatment methods have been developed and studied since the proposed use of lignocellulosic feedstocks for biofuel industries, but all pretreatments attempt to accomplish one or more of the following to improve fermentable sugar yields:

- A reduction in the size of biomass for increased surface area to volume ratio
- The destabilization of the plant structure by removing key constituents (e.g. lignin or hemicellulose)
- An increase of the biomass porosity to facilitate hydrolysis by cellulolytic enzymes

Conventionally, pretreatment methods fall into one of three categories (physical, chemical, and biological), but recent studies often focus on hybrid methods that incorporate the advantages of each approach [8]. This chapter seeks to summarize state-of-the-art pretreatment methods for the conversion of lignocellulosic biomass into biofuels. Optimal sugar and biofuel yields are reported when possible, especially for the production of liquid biofuels. The chapter also briefly discusses the pretreatment options for gaseous biofuel such as biogas generation from biomass.

2 Acid Pretreatments

Dilute acid solutions solubilize the hemicellulosic fractions of second generation feedstocks, thereby improving its vulnerability to cellulolytic enzymes. Since hemicellulose is comprised of a mixture of five and six carbon sugars, the sugar-rich hydrolysate can serve as a substrate for multiple processes such as fermentation (for increase biofuel production), anaerobic digestion (for biogas production), or biochemical/bio-based product production (Fig. 2).

The pretreatment of lignocellulosic biomass with dilute acid solutions is one of the most frequently investigated and applied methods. In a comparative study conducted in 2005, it was suggested that dilute sulfuric acid pretreatment may be the most economically viable pretreatment strategy for improving the conversion of lignocellulosic biomass to ethanol [9]. Since then, a number of studies have sought to improve this technology.

Hsu et al. [10] examined the effects of dilute sulfuric acid on rice straw containing 36.6% glucose, 16.1% xylose, and 14.9% lignin at various acid concentrations of 0.5–1.0% w/w and times of 1–25 min. Following enzymatic hydrolysis, the study reported a maximum total sugar yield of 83% under the optimal conditions: 1% w/w sulfuric acid, 1–5 min, at 160–180°C. Monomeric sugars, furfural, and 5-hydroxymethyl furfural (HMF) were quantified by high pressure liquid chromatography (HPLC). Furfural and HMF are formed by the degradation of xylose and glucose, respectively, and subsequently reduce the ethanol yield. These compounds are typically referred to as inhibitors as they affect the efficacy of sugar-liberating enzymes (cellulases) and ethanol-producing yeast. Hsu et al. found that as the severity of the dilute acid pretreatment increased (on the basis of acid concentration, temperature, and time) the concentration of inhibitors followed correspondingly.

Li et al. [11] found similar results with the use of dilute sulfuric acid at a 1 and 3% w/w solid loading of switchgrass. Amorphous sugar structures, like hemicelluloses, were solubilized from the solid fraction during pretreatment. The results obtained were compared to samples pretreated with an ionic liquid (1-ethyl-3-methylimidazolium acetate). Although the total plant-to-sugar process time was significantly reduced (72–15 h) with a corresponding increase in sugar yield and biomass digestibility for ionic liquid treated samples, the cost of using exotic chemicals (compared to sulfuric acid) may be unjustifiable in the commercial scale.

Advances in technology have allowed for an improved understanding of the dilute acid pretreatment of lignocellulosic biomass. Pingali et al. [12] applied small-angle neutron scattering (SANS) to switchgrass in an effort to understand the morphological changes that occur at various levels of magnification. The results of the study provided insight into several interesting concepts. First, lignin did not seem to redistribute in the plant structure with presence of acid alone; an increased temperature was also required. Based on the transition of lignin, the authors proposed a temperature threshold (between 125 and 192°C) that may be related to the redistribution of lignin phenomena observed in many dilute acid studies. Pingali et al. [12] also noted an observed increase in the crystallinity of cellulose from switchgrass following the removal of hemicellulose via acid. It was proposed that in situ, crystallinity is limited by competition for hydrogen bonding by lignin, hemicellulose, and cellulose. By removing hemicellulose, the authors suggest that the remaining glucan is able to stabilize.

Other innovations have been demonstrated by applying a biorefinery concept to current technology. In conventional processes, lignocellulosic biomass is dried prior to chemical pretreatments for better storage and ease of handling. However this, removes the potential from generating high value co-products from feedstocks with high moisture content. Takara and Khanal [13] considered the use of a tropical, perennial grass (*Pennisetum purpureum* or banagrass) for biofuel production in Hawaii. Using a commercial screw-press, nearly 20% of the moisture was removed from the feedstock as a nutrient-rich effluent for value-added processing. The remaining wet solids were pretreated with dilute acid (5% v/v sulfuric acid) and compared to dried samples. Notably, following enzymatic saccharification, sugar

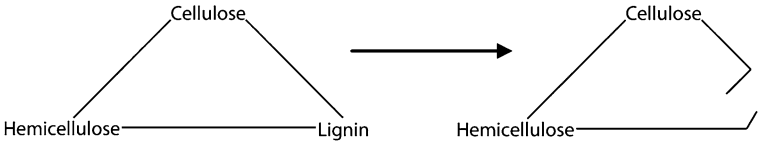

Fig. 3 Simplified schematic of plant composition after alkaline pretreatment

yields obtained from the wet samples were higher (theoretical xylose and ~85% glucose) than that obtained from dried samples (Takara and Khanal [13]).

3 Alkaline Pretreatments

In contrast to acid, aqueous alkaline solutions solubilize and remove the lignin fraction of lignocellulosic biomass. The remaining structure of the feedstock is destabilized and composed mainly of sugar-rich hemicellulose and cellulose solids, which are subsequently saccharified to fermentable sugars via enzymes called (hemi)cellulases (Fig. 3).

Chu et al. [14] confirmed the solubilization of lignin with 10% w/w sodium hydroxide using Raman confocal microscopy. Control samples of *Miscanthus x giganteus* were found to harbor lignin and cellulose within the cell wall, but following pretreatment, only cellulose remained intact and undisturbed. Hemicellulose was difficult to detect with this method as a result of its amorphous structure.

Gupta and Lee [15] compared the effects of ammonia and sodium hydroxide solutions on switchgrass, a perennial model feedstock. Both pretreatments have inherent advantages that correlate to specific feedstocks. Aqueous ammonia, for example, has been known to selectively target lignin in non-woody crops [16], while sodium hydroxide has been particularly useful on woody crops. Gupta and Lee [15] demonstrated that when supplemented with hydrogen peroxide (H_2O_2), both ammonia and sodium hydroxide improved the digestibility of the cellulose to over 90%. Interestingly, however, when hydrogen peroxide (H_2O_2) was applied to ammonia, sugar degradation was increased, whereas when applied to sodium hydroxide, sugar degradation during pretreatment was decreased. The authors suggest that the mechanism in which the alkali dissolves the lignin varies between chemicals. Aqueous ammonia pretreatments selectively react with lignin compared to the carbohydrate and lignin reactions of sodium hydroxide.

Studies comparing the efficacy of two leading chemical pretreatments, ammonia fiber expansion (AFEX) and dilute sulfuric acid, were conducted by Lau et al. [17] using corn stover as a feedstock. AFEX utilizes the low volatility and alkaline properties of anhydrous ammonia to create a combined chemical and physical disruption of the lignin, hemicellulose, and cellulose components of lignocellulosic biomass. The advantages of this approach include lower operating

temperatures, ammonia recovery, faster pretreatment times, and no washing [18]. Lau et al. [17] reported that while 10% of the sugars (particularly xylose) were degraded during dilute acid pretreatments, all of the carbohydrates were preserved when AFEX was applied. Primary drawbacks for AFEX pretreatment, however, are the inherent need for costly hemicellulases to solubilize non-glucose sugars from the solid hemicellulose fraction, and the need for microbes capable of fermenting complex sugar streams to biofuel. Moreover, ammonia recovery processes require additional equipment and infrastructure. In contrast to alkaline pretreatments, dilute acid requires less equipment, and hydrolyzes the five carbon sugar backbone of most hemicellulosic structures into soluble monomers.

4 Biological Pretreatments

The biological degradation of lignocellulosic material may be the oldest form of pretreatment available for effectively accessing the five and six carbon sugars of plant structures. Microorganisms, like fungi, excrete specific enzymes (ligninase) to dissolve the protective lignin coating of biomass, making sugars like glucose and xylose available for consumption. In the past decade, many studies focused on the potential that these pretreatments had on the biofuel industry, but in recent years, it has become clear that biological methods alone are too slow to be economically viable [7].

Brown rot fungi were considered for the biological pretreatment of softwood for improved glucose release, and subsequently biofuel production. Ray et al. [19] exposed pine sapwood to two species of brown rot fungi (*Coniophora puteana* and *Postia placenta*) for 20 and 25 days, respectively, before conducting preliminary fermentation studies. Cellulose to glucose yields were reported to be about 70%, suggesting relatively high efficiency for this type of process. Pretreatment, however, required a minimum of two weeks, with the additional need for enzymatic saccharification and fermentation. The feasibility of fungal pretreatment thus may not be viable in commercial-scale applications.

Yang et al. [20] reported on the use of a thermophilic bacterium, *Anaerocellum thermophilum*, for ethanol production from untreated lignocellulosic biomass. Poplar and switchgrass were used as substrates to cultivate the bacterium at 75°C until a stationary growth phase was observed. Despite achieving high cell densities within 20 h (much faster than fungal cultures), ethanol was not detectable in the product stream.

5 Fractionation

The term fractionation is used frequently in biomass-to-biofuel conversion processes, but is typically ill-defined. Fractionation generally refers to the separation and isolation of various components of the entire feedstock, with the intent of

using each respective component for the generation of either biofuels or bio-based products. In practice, nearly all pretreatments can be considered to fractionate lignocellulosic biomass, but more commonly, the term fractionation is coupled with solvent-type pretreatments.

Although phosphoric acid is technically a mineral acid, its use is classified as a solvent-type pretreatment rather than chemical pretreatment, since it employs organic (solvent) compounds like acetone during biomass washing steps. Moxley et al. [21] examined the effects of phosphoric acid on the cellulose digestibility of industrial grade hemp, and considered its application as a multi-feedstock pretreatment strategy. Previous studies with conventional feedstocks like corn stover, switchgrass, and poplar, suggested that the key parameters to successful sugar release were the acid concentration, temperature, and residence time [22]. The authors defined a critical acid concentration of 83%, after which the effects of phosphoric acid on the hemp structure became prevalent. Maximum cellulose digestibility (96%) was achieved after pretreatment with 84% phosphoric acid at 50°C for 60 min, and enzymatic saccharification for 24 h with a 15 filter paper unit (FPU) per glucan enzyme dosage.

In 2010, a similar approach using phosphoric acid was examined by Sathitsuksanoh et al. [23] for bamboo; a C_4 perennial feedstock. Using low enzyme dosages (1 FPU) following saccharification, the authors were able to achieve 88.2% cellulose digestibility within 72 h, corresponding to a 15-fold reduction in enzyme loadings. Lower required enzyme concentrations have the potential to reduce production costs significantly, and a rigorous techno-economic analysis may help to determine the full extent of the cost savings. Other recent studies have confirmed the results obtained by phosphoric acid pretreatment at critical conditions [2, 24].

6 Hot Water Pretreatment

Hot water washing is an appealing lignocellulosic pretreatment due to its independence from caustic chemicals and benign environmental impacts. Hot water, typically referring to pressurized water maintained above its boiling temperature, is known to promote the hydrolysis of hemicellulosic and amorphous glucose in biomass structures. As part of a Consortium for Applied Fundamentals and Innovation (CAFI), researchers at various institutions compared the yields obtained for the conversion of corn stover to soluble sugars, and subsequently ethanol [25]. Hot water washing was demonstrated to solubilize 90% of the cellulose to glucose with a 15 FPU/g glucan enzyme loading after being pretreated at 190°C, 15 min, and 16% solid-to-water ratio [25]. When a similar pretreatment was applied to wheat straw, lower yields were obtained. Hemicellulosic sugars from wheat straw were reported to be best removed at temperatures around 184°C (71.2% recovered), while cellulose was best removed after pretreatment at 214°C and enzymatic hydrolysis (90.6% recovered); thus suggesting a two-stage hot water approach [26]. For rice straw, yields of 85% glucose (after enzymatic

hydrolysis) and sugar degradation were witnessed when pretreated with water at 180°C and above [27].

Hot water washing can be incorporated into flow-type reactors, improving hemicellulose (from 60 to 90%) and lignin (30–75%) removal. Pronyk and Mazza [28] evaluated the use of high pressure (11 MPa) water in a custom-designed reactor on the cereal crop, triticale. Optimal conditions were determined by the maximum sugar released from hemicellulose, which occurred at 165°C, 115 mL/min, and 60 mL/g solvent-to-solid ratio. The release of glucose from cellulose was reported to be highly temperature dependent, and 90% digestibility yields (following enzyme hydrolysis) were not achieved below temperatures of 170°C.

Sorghum bagasse in a flow-type reactor produced similar results. The hot water was supplemented with metal salts to increase the rate of cellulose and hemicellulose decomposition. With 0.1% copper dichloride, total sugar digestibility was reported to improve from 75.2 to 90.4% [29].

7 Steam Explosion

Similar to hot water washing, steam explosion pretreatments employ the properties of water to facilitate the deconstruction of lignocellulosic feedstocks. Unlike the previously described methods, steam explosion by itself, however, produces comparatively low sugar yields; often around 70% under optimal conditions [30, 31]. Most steam explosion techniques are supplemented with acid catalysts (like sulfuric acid) or conducted immediately following chemical pretreatments [32]. Like AFEX, high pressure is maintained in a reactor vessel for a predetermined duration before undergoing rapid decompression. The rapid change in pressure weakens the cellulosic fibers, subsequently improving enzymatic saccharification to glucose.

8 Physical Pretreatment

Physical pretreatment describes the purely mechanical destruction of lignocellulosic biomass. Such strategies employ the use of cutting, ball, and hammer mills, as well as screw presses and ultrasound. Although the term is still widely used, it is generally understood that nearly all feedstocks require some sort of size reduction (e.g. physical pretreatment) after field harvests.

In cases where the moisture content of the raw material is high, (>60% w/w) however, unconventional physical pretreatments, like mechanical extrusion, may be implemented both to facilitate handling and storage, and generate a liquid substrate for co-product generation [13]. Studies examining the use of screw presses (often referred to as extrusion) have markedly shown improvement in the pretreatment and processing of lignocellulosic material both into fermentable sugars [13] and biogas [33]. In the case of biogas, yields of methane increased by

as much as 18–70% [33] supporting its incorporation into a renewable energy economy. Extrusion technology has been commercially implemented in Europe for biomass pretreatment for biogas production and is a form of thermomechanical communition that promotes the disintegration of plant materials by means of mechanical energy resulting from friction, squeezing, and crushing. Extrusion increases the surface area by as much as 75% [34]. In addition, extrusion also helps to break-up the wax layer in green (young) plants.

9 Challenges and Economics

Technology and innovation in the field of biofuels continue to excel rapidly, rendering most critical literature reviews obsolete prior to publication, but to date, the challenge of converting second generation lignocellulosic feedstocks to biofuel *economically* remains to be fully addressed. Harvesting, transporting, storing, preprocessing, pretreating, and saccharifying raw materials are just a few examples of unit operations that contribute significantly to overall costs before fermentation and distillation. Careful planning and design of biofuel facilities, however, can reduce the production costs with little technological advancements. For example, Kocoloski et al. [35] reported that strategic placement of biofuel facilities in locations that reduce the transportation of feedstock and product has the potential of saving $0.20–0.30 per gallon of biofuel.

As part of the CAFI study conducted in 2005, a techno-economic analysis by Eggeman and Elander [9] suggested that dilute acid pretreatment was the most viable for reducing lignocellulosic recalcitrance; yielding an ethanol value of about $5.13–8.72 per gallon gasoline equivalent from corn stover [1]. Production costs are often based on models established by the National Renewable Energy Laboratory (NREL) [1, 35, 36] and assume the dilute acid pretreatment of corn stover or switchgrass. In most cases, the costs of producing biofuel from a single crop are inherently reliant on feedstock prices, which depending on geography and available resources (e.g. water), may be volatile. Attempts to produce biofuel at lower costs have, in some cases, met with success, but large-scale production at commercial volumes remains uncompetitive with gasoline to date.

10 Future Work

Technological advances to improve the efficiency of lignocellulosic conversions are continually developed throughout the world, but more recently, an emphasis has been placed on a biorefinery concept which parallels its petroleum predecessors by fractioning a single feedstock into multiple products. It has become evident that in the long-term future, the replacement of fossil fuel brings the inherent challenges of finding renewable alternatives for replacing petrochemical-derived

compounds such as plastics and other polymers. Thus there exists an urgent need to produce biochemicals and bio-based products concurrently with biofuels.

The primary focus of this chapter has been on state-of-the-art pretreatment technologies of lignocellulosic materials rather than its production and use, but it would be prudent to briefly summarize some of the advances made in the transportation fuel sector. Europe, in particular, can be considered to be years ahead of the US in transportation fuel technology. Because biomass feedstock costs can be volatile like the price of oil, much attention has been placed on gaseous fuel (upgraded biogas or methane) produced from solid and liquid waste streams, perennial grass, energy crops, and agri-residues. Biogas, produced via anaerobic digestion, consists of 50–75% methane [37], and can be produced in decentralized and centralized locations. Countries like Sweden have pioneered the use of upgrading biogas as a light duty vehicle fuel, and the consumption of biogas in the country has already surpassed natural gas [38]. Similar efforts are also being made in Germany which currently operates nearly 5,000 anaerobic digesters for bioenergy production [34]. In the UK, concerns over the use of biogas as fuel stem from a lack of quality standards and infrastructure, as well as competition with other uses of biogas. For example, combined heat and power (CHP) generation may be more economical for using biogas rather than in vehicles [39]. While the ultimate fate of biogas as a renewable fuel is still relatively unknown in the United States, in the near future, there will likely be a push for policies and subsidies to improve the economic competitiveness of these technologies with natural gas and fossil-derived fuels.

11 Conclusion

Advances in technology have given rise to a wide breadth of pretreatment strategies that cannot fully be covered in a single chapter. Despite their multitude of differences, however, the underlying theme of all biomass pretreatments remain the same: reduce the recalcitrance of the feedstock to facilitate the bioconversion of (hemi)cellulose into fermentable sugars and ultimately, biofuel.

With the advent of the biorefinery perspective, the concept of fractionation and maximizing revenue-generating bio-based products from a single feedstock has facilitated innovation in biofuel production. Whether the future relies on gaseous or liquid-based fuels or bosssth is yet to be determined, but likely, region-specific crops will promote the decentralization of commercial-scale industries and advocate the development of region-specific bio-based products. Thus any or all of the pretreatment methods presented above may move to commercial scale to meet demands in various regions of the world.

References

1. Kazi FK, Fortman JA, Anex RP et al (2010) Techno-economic comparison of process technologies for biochemical ethanol production from corn stover. Fuel 89:S20–S28
2. Rollin JA, Zhu Z, Sathitsuksanoh N et al (2011) Increasing cellulose accessibility is more important than removing lignin: a comparison of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia. Biotechnol Bioeng 108:22–30
3. Mueller SA, Anderson JE, Wallington TJ (2008) Impact of biofuel production and other supply and demand factors on food price increases in 2008. Biomass Bioenerg. doi:10.1016/j.biombioe.2011.01.030
4. Ajanovic A, Haas R (2010) Economic challenges for the future relevance of biofuels in transport in EU countries. Energy 35:3340–3348
5. Liu Z, Saha BC, Slininger PJ (2008) Lignocellulosic biomass conversion to ethanol by *Saccharomyces*. In: Wall J, Harwood C, Demain A (eds) Bioenergy. ASM Press, Washington
6. Amthor JS (2003) Efficiency of lignin biosynthesis: a quantitative analysis. Ann Bot-Lond 91:673–695
7. Shrestha P, Rasmussen M, Khanal SK et al (2008) Solid-substrate fermentation of corn fiber by *Phanerochaete Chrysosporium* and subsequent fermentation of hydrolysate into ethanol. J Agr Food Chem 56:3918–3924
8. Takara D, Shrestha P, Khanal SK (2010) Lignocellulosic biomass pretreatment. In: Khanal SK (ed) Biofuel and bioenergy from biowastes and lignocellulosic biomass. American Society of Civil Engineers, Reston
9. Eggeman T, Elander RT (2005) Process and economic analysis of pretreatment technologies. Bioresource Technol 96:2019–2025
10. Hsu TC, Guo GL, Chen WH et al (2010) Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis. Bioresource Technol 101:4907–4913
11. Li C, Knierim B, Manisseri C et al (2009) Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. Bioresource Technol 101:4900–4906
12. Pingali SV, Urban VS, Heller WT et al (2010) Breakdown of cell wall nanostructure in dilute acid pretreated biomass. Biomacromolecules 11:2329–2335
13. Takara D, Khanal SK (2011) Green processing of tropical banagrass into biofuel and biobased products: an innovative biorefinery approach. Bioresource Technol 102:1587–1592
14. Chu LQ, Masyuko R, Sweedler JV et al (2010) Base-induced delignification of *Miscanthus x giganteus* studied by three-dimensional confocal Raman imaging. Bioresource Technol 101:4919–4925
15. Gupta R, Lee YY (2010) Investigation of biomass degradation mechanism in pretreatment of switchgrass by aqueous ammonia and sodium hydroxide. Bioresource Technol 101:8185–8191
16. Kim TH, Kim JS, Sunwoo C et al (2003) Pretreatment of corn stover by aqueous ammonia. Bioresource Technol 90:39–47
17. Lau M, Gunawan C, Dale B (2009) The impacts of pretreatment on the fermentability of pretreated lignocellulosic biomass: a comparative evaluation between ammonia fiber expansion and dilute acid pretreatment. Biotechnol Biofuels 2:30
18. Teymouri F, Laureano-Perez L, Alizadeh H et al (2005) Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Bioresource Technol 96:2014–2018
19. Ray MJ, Leak DJ, Spanu PD et al (2010) Brown rot fungal early stage decay mechanism as a biological pretreatment for softwood biomass in biofuel production. Biomass Bioenerg 34:1257–1262
20. Yang SJ, Kataeva I, Hamilton-Brehm SD et al (2009) Efficient degradation of lignocellulosic plant biomass, without pretreatment, by the thermophilic anaerobe "*Anaerocellum thermophilum*" DSM 6725. Appl Environ Microbiol 75:4762–4769

21. Moxley G, Zhu Z, Zhang YHP (2008) Efficient sugar release by the cellulose solvent-based lignocellulose fractionation technology and enzymatic cellulose hydrolysis. J Agr Food Chem 56:7885–7890
22. Zhang YHP, Ding SY, Mielenz JR et al (2007) Fractionating recalcitrant lignocellulose at modest reaction conditions. Biotechnol Bioeng 97:214–223
23. Sathitsuksanoh N, Zhu Z, Ho TJ et al (2010) Bamboo saccharification through cellulose solvent-based biomass pretreatment followed by enzymatic hydrolysis at ultra-low cellulase loadings. Bioresource Technol 101:4926–4929
24. Zhu Z, Sathitsuksanoh N, Vinzant T et al (2009) Comparative study of corn stover pretreated by dilute acid and cellulose solvent-based lignocellulose fractionation: enzymatic hydrolysis, supramolecular structure, and substrate accessibility. Biotechnol Bioeng 103:715–724
25. Mosier N, Hendrickson R, Ho N et al (2005) Optimization of pH controlled liquid hot water pretreatment of corn stover. Bioresource Technol 96:1986–1993
26. Pérez JA, Ballesteros I, Ballesteros M et al (2008) Optimizing liquid hot water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production. Fuel 87:3640–3647
27. Yu G, Yano S, Inoue H et al (2010) Pretreatment of rice straw by a hot-compressed water process for enzymatic hydrolysis. Appl Biochem Biotech 160:539–551
28. Pronyk C, Mazza G (2011) Optimization of processing conditions for the fractionation of triticale straw using pressurized low polarity water. Bioresource Technol 102:2016–2025
29. Yu Q, Zhuang X, Yuan Z et al (2011) The effect of metal salts on the decomposition of sweet sorghum bagasse in flow-through liquid hot water. Bioresource Technol 102:3445–3450
30. Lu Y, Wang Y, Xu G et al (2010) Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass. Appl Biochem Biotech 160:360–369
31. Yang J, Zhang X, Yong Q et al (2010) Three-stage hydrolysis to enhance enzymatic saccharification of steam-exploded corn stover. Bioresource Technol 101:4930–4935
32. Chen WH, Pen BL, Yu CT et al (2011) Pretreatment efficiency and structural characterization of rice straw by an integrated process of dilute-acid and steam explosion for bioethanol production. Bioresource Technol 102:2916–2924
33. Hjorth M, Gränitz K, Adamsen APS et al (2011) Extrusion as a pretreatment to increase biogas production. Bioresource Technol 102:4989–4994
34. Effenberger M, Andrade D, Bachmaier H et al (2011) Grass fermentation technology: equipment, fermentation process, carbon footprint. In: 20th Biogas annual conference and biogas trade, 2011 Nuremberg, Germany, pp 29–37
35. Kocoloski M, Michael Griffin W, Scott Matthews H (2011) Impacts of facility size and location decisions on ethanol production cost. Energ Policy 39:47–56
36. Leboreiro J, Hilaly AK (2011) Biomass transportation model and optimum plant size for the production of ethanol. Bioresource Technol 102:2712–2723
37. Poeschl M, Ward S, Owende P (2010) Prospects for expanded utilization of biogas in Germany. Renew Sust Energ Rev 14:1782–1797
38. Börjesson P, Mattiasson B (2008) Biogas as a resource-efficient vehicle fuel. Trends Biotechnol 26:7–13
39. Patterson T, Esteves S, Dinsdale R et al (2011) An evaluation of the policy and techno-economic factors affecting the potential for biogas upgrading for transport fuel use in the UK. Energ Policy 39:1806–1816

Second-Generation Biofuel Production from Corn-Ethanol Industry Residues

Prachand Shrestha, Anthony L Pometto III, Samir Kumar Khanal and J. (Hans) van Leeuwen

Abstract Net ethanol production per unit mass of corn kernel can be significantly improved by utilizing fibrous co-products to produce cellulosic ethanol. Corn fiber is a good cellulosic feedstock to produce second-generation biofuel. A biorefinery concept is introduced to convert fibrous residue, corn fiber, into fermentable sugars at a lower temperature with minimal use of chemicals. Laboratory-scale consolidated fermentation system comprised of on-site fungal enzyme production system and simultaneous saccharification and fermentation (SSF) yielded 7.1, 8.6 and 4.1 g ethanol per 100 g corn fiber when saccharified with the white-, brown-, and soft-rot fungi, respectively. The highest corn-to-ethanol yield (8.6 g ethanol/100 g corn fiber) was equivalent to 42% of the theoretical ethanol yield from starch and cellulose in corn fiber. This is equivalent to 120 l of ethanol per metric ton of corn fiber. With process optimization, conversion of over 70% of corn fiber carbohydrate content into ethanol can generate as much as $13x10^9$ l of ethanol per year, which is equivalent to 25% of the current annual ethanol production ($52x10^9$ l) in the US, additional $8.65 billion annual revenue and reduction in corn acreage by 3 mha. It is also possible to convert the carbohydrates to a fuel oil using a

P. Shrestha (✉)
Energy Biosciences Institute (EBI), University of California,
Berkeley, CA 94720, USA
e-mail: prachand@berkeley.edu

A. LP. III
Food Sciences and Human Nutrition, Clemson University, SC 29634, USA

S. K. Khanal
Molecular Bioscience and Bioengineering, University of Hawaii at Manoa,
Honolulu, HI 96822, USA

J. (Hans)van Leeuwen
Civil, Construction and Environmental Engineering, Iowa State University,
Ames, IA 50011, USA

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*,
Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_5,

secondary oleaginous fungal process. The residual fiber enriched with fungal protein can still be utilized as animal feed without unbalancing the feed market/supply.

1 General Introduction

Gross domestic product (GDP) is an indicator for a nation's socio-economic development and is correlated with energy consumption [7]. Most recently, we have experienced the shift of industrial development from developed nations to many developing nations like China and India. For example, the growth in energy demand in Chinese industries has increased the outputs and henceforth, has improved China's GDP [25]. These countries consumed 20% of the world's total energy consumption (495 quadrillion BTU) in 2007 [8]. It has been projected that by 2035 the non-OECD (Organization for Economic Cooperation and Development) countries including China and India will increase their energy consumption rate to 30% of the world's energy consumption. Likewise, socioeconomic status and purchasing capacities of people in these countries have also improved in recent years. This further demands various forms of energy (coal, natural gas, petroleum), food and natural resources (to provide raw materials for industries). Nonrenewable resources have dramatically changed our environment by increasing emission of CO_2-a greenhouse gas (GHG). Utilization of energy efficient technologies, reduction in GHG emission and exploration of renewable energy resources can significantly mitigate the alarming concerns of energy, economy and environment.

Johansson et al. [14] further addressed that opting energy efficiency strategy could not alone resolve the energy demands of all countries in the world. However, utilization of renewable energy resources (e.g. biomass, solar, wind, hydel, geothermal) could contribute to a greater portion of the energy demand problem and may replace up to 40% of the energy demand by the middle of the twenty first century. Rosen [23] suggested that energy efficiency is further required for using sustainable energy resources. By 2030, the US aims to replace up to 20% of its transportation fuel and 25% of industrial energy needs from biomass resources [35]. Currently, plant derived biomass energy accounts for 9–13% total global energy supply [34]. Biomass has diverse applications such as producing heat, electricity (e.g. gasification) and liquid biofuel (e.g. ethanol, butanol, aviation fuel etc.) and gaseous biofuel (hydrogen, methane etc.). On commercial scale, biomass energy and bio-economy promise sufficient energy supply, rural employment and a closed carbon cycle [10]

The annual global energy demand is about 500 quadrillion BTUs. US, an OECD member, not only consumes the highest amount of supplied global energy i.e., $\sim$100 quadrillion BTUs; but also consumes the highest amount of liquid transportation fuels annually. It is estimated [37] that, by 2035, its annual liquid

consumption will increase to 22.0 million barrels per day from 18.8 million barrels per day in 2009. The majority (~60%) of the fuel demands in USA are fulfilled by petroleum imports from politically unstable Middle East nations. The increasing demands of fuels in countries like China and India and the volatility in recent (summer of 2008) crude oil market also demands a paradigm shift to energy independence for the US. Various alternatives have been sought to manage and stabilize the energy security especially in Europe and the US. Research and development for cost-effective production of biofuels in transportation sector have received greater global attention. Current corn and sugarcane based bio-ethanol production is 50 and 26 x 10^9 l per year in USA and Brazil, respectively [22]. While the production of first generation starch/sugar-based ethanol will continue to expand in foreseeable future, it will be limited by the availability of feedstock, climates for the respective monoculture crops, and increasing concern over food versus fuel supply. Therefore, second generation cellulose-based biofuels, from ubiquitous cellulosic feedstocks (agricultural and forest residues, energy crops, and industrial and municipal wastes), have recently received increasing attention to substantially replace fossil fuel demand in coming decades.

Lignocellulose represents the most abundant polymer in the biosphere (10–50 x 10^9 tons annually) but unlike corn starch it consists of three different polymers lignin, cellulose and hemicellulose [4]. Dale [6] emphasized the biomass refinery approach–holistic usage of all lignocellulosic components and development of economical biomass pretreatment process to provide inexpensive and feasible feedstock. The only common polymer to all plants is cellulose. The lignin and hemicellulose for every plant are unique. Thus, the structural complexity and recalcitrance of lignin and plant cell wall polysaccharides impose inherent challenges to physical, chemical and biological means of depolymerization [15]. Physical and chemical pretreatments enhance the enzymatic conversion [5, 9, 13, 20] but the cost of pretreatment may be as much as 20% of the unit ethanol production cost [41]. Thus, plant biomass pretreatment represents one of the major bottlenecks in biochemical conversion of biomass to biofuel.

Second-generation ethanol production utilizes primarily cellulosic (β1,4-glucose polymer) feedstock, which undergoes pretreatment via physical, chemical or biological means for size reduction, selective removal of plant cell wall components (e.g., lignin) and improvement in enzyme accessibility for hydrolysis followed by enzymatic hydrolysis and simultaneous fermentation of the resulting sugars (C-6 and/or C-5 sugars) to ethanol [16]. Abundance of native feedstock (e.g., USA produces ~ 1.3 x 10^9 metric tons of lignocellulosic biomass annually, USDA-USDOE, [35]) is favorable toward sustainable renewable biofuel generation. In secondary plant cell walls, cellulose is present in network with hemicellulose and lignin, which provides plants with recalcitrant properties to microbial decay and structural rigidity. Therefore, pretreatment and enzyme hydrolysis steps are critical but costly steps during cellulosic bioconversion to biofuel [16]. Chemical (alkali and acid) pretreatment not only generates inhibitory compounds that are detrimental to downstream fermentation, but such pretreatment is also expensive. It is therefore very necessary to reduce chemical cost and

environmental footprints and explore alternative environment friendly and economically sound processes such as direct biological conversion of cellulose to ethanol.

Biological approaches towards cellulose-ethanol research bio-mimic the natural process of wood degradation. Wood itself is a typical example of natural cellulose. Scientists and plant pathologists have been conducting research to explain physiology and mechanism of various types of wood-rot, caused by certain fungi. Earlier studies explored the cause and prevention of fungal deconstruction of wood. Extracellular enzymatic degradation and non-enzymatic oxidative degradation of the substrate were identified as the main wood decay mechanisms. The authors have been involved in research that seeks to examine natural wood-rot degradation mechanism to develop an in situ fungal cellulase/hemicellulase production process to degrade cellulose [27]. We investigated biological pretreatment using white-, brown- and soft-rot fungi in biological pretreatment and hydrolysis of lignocellulose feedstock (wet-milled corn fiber) to produce monomeric sugar that would then be converted to bioethanol (and bio-based product).

The three main research objectives were:

a. Evaluate effectiveness of mild alkali, alkaline peroxide and steam pretreatment of wet-milled corn fiber prior to solid-substrate fermentation by white-, brown- and soft-rot fungi (*Phanerochaete chrysosporium, Gloeophyllum trabeum and Trichoderma reesei,* respectively) and subsequent fermentation of hydrolyzate to ethanol using *Saccharomyces cerevisiae* [29]
b. Evaluate white-, brown- and soft-rot fungi for saccharification of wet-milled corn fiber via enhanced enzymatic hydrolysis, and the subsequent fermentation of fermentable sugars into ethanol using *S. cerevisiae* [28]
c. Use the fermentable sugars produced to synthesize bio-oil via a secondary fungal process [39]

2 Additional Ethanol from Corn-to-Ethanol Plant Residue

The Renewable Fuels Association [22] reported the 204 US-based biorefineries have total annual ethanol production capacity over 52×10^9 l (13.74×10^9 gallons). In addition to ethanol, these industries also produce significant amount of fibrous co-product (i.e., corn fiber), which is currently being incorporated into animal feed. Depending on the type of biorefinery, dry-grind or wet-millinge, the co-product is further processed and sold as distiller's dried grain with solubles (DDGS) or corn gluten feed/meal. Over 32.5 million metric tons (mmt) of fibrous co-products were produced from corn-based ethanol industries in 2010. Last decade (2000–2010), the total ethanol production increased by 11-folds while DDGS generation exceeds sevenfolds (Every bushel i.e., 25.4 kg of corn produces about 8.16 kg of DDGS). Huge quantities of these co-products, which currently suffice the domestic and international feed market, may pose serious management

issues and also saturate existing market (e.g., animal feed) in long run. Additionally DDGS favor ruminant animal feed (cattle and diary) versus monogastric animal feed (swine and poultry). It is also desirable to diversify the R&D prospect of processing these co-products on site, beyond animal feed. For example, ethanol production from these co-products will generate additional revenue. To maximize the fermentation output and generate new revenue sources in corn dry-grind industry, [31] developed a novel system that incorporates initial soaking step, degermination and density separation (prior to enzymatic liquefaction and fermentation in dry-grind ethanol setup) to separate fibrous residue known as quick fiber, which represents 6–7% of yellow dent kernel and about 46–60% of recoverable fiber in hybrid dry-grind processing. This polysaccharide rich quick fiber also contains corn oil with cholesterol lowering components like ferulate phytosterol esters (FPE) and phytosterol components. Therefore, quick fiber can serve as promising feedstock for multiple products–ethanol and nutraceutical rich corn oil. The wet-milled corn fiber contains (on dry matter basis) lignin (2%), cellulose (18%), hemicellulose (35%) and some residual starch (18%) [1]. Conversion of polysaccharide fraction (starch, cellulose, and hemicelluloses) from these components to ethanol would yield an additional 25% of current annual ethanol production capacity (i.e., 13×10^9 l of ethanol). This would fetch over $8.65 billion annually at current ethanol selling price of 66 cents per l ($2.5 per gallon ethanol) and also minimizes additional corn usage for ethanol production. The extra ethanol production is equivalent to 7.4×10^6 acres. According to the National Corn Growers Association in the USA the average corn production per acre was 153.9, 164.9, and 152.8 bushels in 2008, 2009, and 2010, respectively, with an average price per bushel of $3.90, $3.70, and $5.30 (www.ncga.com). Corn fiber from the wet- and dry-grind process is essentially collected and pretreated lignocellulose feedstock compared to other plant sources as it has been cleaned, ground, and hot water extracted. Corn fiber, therefore, could serve as a model cellulosic feedstock for cellulosic ethanol production. Additionally, the lignin content is also low in corn fiber residues. Therefore, inclusion of fiber (with residual starch and holocellulose) conversion into ethanol can improve net ethanol production per unit weight of corn by 13% [17]. Second-generation biofuels, from conversion of residues like corn fiber and corn harvesting leftovers like stover, are necessary to sustain the net productivity and profitability of corn-based ethanol biorefineries. Ethanol industry is gearing up for cellulosic ethanol. POET is pursuing to utilize 770 tons per day of cellulosic residues e.g. corn cobs and husks for ethanol production (Project Liberty, [19]). Research is currently focused on utilizing significant amounts of cellulosic biofuels from corn stover without jeopardizing its usage to control soil erosion and soil health. Conversions of cleaner cellulosic residues like corn fiber, corn cobs and hardier material such as corn stover can substantially add volumetric second generation biofuels to suffice the target of 136×10^9 l (36 billion gallons) of cellulosic biofuels by 2022.

3 Technological Hurdles in Cellulose Biofuel Production

Unlike amylose (linear starch with α-1.4-glyosidic bonds) and amylopectin (branched starch with α-1,4- and α-1,6-glycosidic bonds) produced in grains (e.g. corn, wheat, rice, sorghum) lignocellulose consists of cellulose (linear β-1,4-glyocside bonds), hemicellulose (various combination of C-5 and C-6 sugars and glycoside bonds), and lignin (recalcitrant phenylpropane polymer), which represents the glue between cell walls. Thus, the molecular bondings and structural complexity of hemicelluloses and lignin in lignocelluloses provide structural rigidity, resistance to microbial attacks and water insolubility. Thereby the recalcitrant nature of lignocellulose poses hindrance to chemical and biological breakdown. Bioconversion of lignocelluloses requires combinations of pretreatments which include size reduction, chemical pretreatments and structure alterations to remove lignin which may require extreme temperature and pressure regimes. These pretreatments are then followed by extensive water extractions to remove all enzymatic and microbial inhibitors (usually alkaline soluble lignin and phenolics). Once thoroughly washed a series of enzyme hydrolysis steps are required in order to breakdown the complex water insoluble cellulose and hemicellulose molecules into fermentable sugars (mono- and di saccharides [24].

The biomass pretreatment and enzymes required for hydrolyzing polysaccharides are essential but costly steps involved in biofuel production from lignocellulosic biomass. To date, there is not a single large-scale (mega-million gallon capacity) lignocellulose biorefinery in operation. Many of the small-scale demonstration plants are funded through federal research grants. One of the primary reasons is the high cost of chemical (alkali and acid) pretreatment and removal of microbial and enzymatic inhibitory compounds consumes large quantities of water and generates large volumes of waste [12]. In the mean time, the unit cost on enzyme dosage (i.e., enzyme cost in dollars per gallons of biofuel (ethanol) produced is still very costly. Many enzyme industries like Novozymes, Genencor and DSM are supported by federal grants to conduct detail research and significantly improve the activities of microbial enzymes that can be used to hydrolyze cellulose and hemicellulose from different types of lignocellulosic feedstock [3]. There have been significant increases in catalytic activities of these industrial enzymes but cost associated with volumetric large-scale applications (dosages) of these enzymes for economical cellulosic biofuel production is a major concern. Thus research universities, national laboratories, and biotechnology industries throughout the world are exploring various natural sources such as tropical rain forests, hot springs, termite guts, cow rumen or culture collections for microbial enzymes that can significantly degrade cellulosic feedstock under desired environmental conditions. Needless to mention, volumetric ethanol concentrations from cellulosic feedstock are still very low compared to starch or sucrose based ethanol, and this also increases distillation costs significantly.

4 Conversion of Corn Fiber into Ethanol through Fungal Bioprocessing

Structural plant tissues such as cellulose fibers have been target feedstock to produce renewable biofuel such as ethanol for decades. Wiegel [40] reported a two-stes process of converting cellulose to ethanol (1) hydrolysis of the polysaccharides and (2) fermentation of glucose to ethanol using yeast, *S. cerevisiae*.

The author also suggested the possibility of directly converting the cellulose to ethanol using bacteria like *Clostridium thermocellum*. There has also been a school of thought regarding conversion of both five- and six-carbon sugar into ethanol.

Singh et al. [30] discussed the potential application of filamentous fungal species of the genera *Fusarium*, *Monilla* and *Neurospora* for production of extracellular enzymes for hemi/cellulose hydrolysis and fermentation of glucose and xylose to ethanol. However, ethanol and sugar tolerance by these fungi is lower and sugar to ethanol conversion is slower compared to yeast. South et al. [33] conducted simultaneous saccharification and fermentation (SSF) of acid pretreated hardwood flour via supplementation of cellulase from *T.* reesei and *β*-glucosidase and *S. cerevisiae* to convert glucose to ethanol. The authors also reported direct microbial conversion of acid pretreated hardwood flour to ethanol using *C. thermocellum*. So and Brown [32] compared the Waterloo fast pyrolysis process followed by fermentation process with SSF and dilute acid hydrolysis and fermentation in terms of ethanol production cost for a hypothesized 25 million gallon per year (MGY) cellulosic ethanol refinery. The unit production cost of ethanol via fast pyrolysis and fermentation was reported to be slightly higher than SSF and dilute acid hydrolysis and fermentation process. The authors suggested further feasibility research on pyrolysis coupled with ethanol production and the recovery of lignin as a value-added product to minimize the unit cost of ethanol production. Lignocellulosic ethanol bioprocessing still has big challenges to overcome, especially the high cost of converting lignocellulose to a fermentable sugar mixture. For the optimization of the (ligno-) cellulosic ethanol process, it is also very necessary to first understand the morphological, anatomical and physiological characteristics of each of the plant cell wall tissues that pose recalcitrant and robust structural assemblies against degradation [11]

Corn fiber contains (on dry-matter basis) ~ 70% sugar compounds [28]. Thus, it makes sense to reprocess this co-product into more ethanol. A successful biomimicry concept was developed for efficient conversion of lignocellulosic biomass such as corn fiber and corn stover into ethanol by using wood-rot fungi–the molds which have, otherwise, been studied for damage of commercial timber and in forest pathology. We investigated white- and brown-rot fungi, those that decay wood logs and litter in the forest to recycle carbon and nutrients from lignin, cellulose and hemicellulose. They produce extracellular enzymes like ligninase, cellulase and hemicellulase to degrade these complex polymers. Utilization of

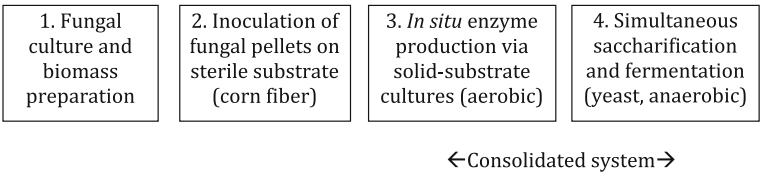

Fig. 1 Bench-scale consolidated bioprocessing of corn fiber into ethanol (adapted from [26]

their extracellular enzyme for degradation of lignocellulose co-products like corn fiber would provide simple sugars and these can be fermented further to ethanol.

In situ extracellular enzyme secretion by these wood-rot fungi can eliminate:

(a) pretreatment cost of lignocellulose degradation, and (b) enzyme cost.

This chapter briefly describes protocols that employ two wood-rot fungi: *Phanerochaete chrysosporium* (white-rot fungus) and *Gloeophyllum trabeum* (brown-rot fungus) in bench-scale solid-substrate fermentation, a process that mimics natural microbial decay of plant matter, to induce cellulosic hydro-lyzing enzymes. Moist wet-milled corn fiber was inoculated with a wood-rot fungus to produce in situ lignin and cellulose degrading enzymes. Within three to five days, they produce considerable amounts of these hydrolytic enzymes that hydrolyze complex polysaccharides into fermentable sugars. For the first time, we reported that these fungi were able to produce ethanol without prescribed conditions [21, 26, 28, 29]. Laboratory scale (1-l experiments), produced 120 l of ethanol per metric ton of wet-milled corn fiber without any biomass pretreatment and addition of commercial enzymes.

5 Conversion of Corn Fiber to Ethanol in Consolidated Fungal Bioprocessing

The two wood-rot fungi: *P. chrysosporium* (white-rot fungus) and *G. trabeum* (brown-rot fungus) were studied in bench-scale solid-substrate fermentation [20, 26], where a predetermined amount of wet-milled corn fiber was inoculated with calculated volume of fungal pellet slurry (Fig. 1). Solid-substrate cultures of these fungi on corn fiber (37°C aerobic condition, 1–5 days) confirmed lignocellulose degrading activities as determined by specific hemi/cellulase enzyme activity and Klason lignin assays. The ethanol yields from the consolidated fungal process respectively resulted in 3 and 4 g ethanol per 100 g of corn fiber for white- and brown-rot fungal system. These fungi were also able to ferment sugar to ethanol without addition of yeast during SSF. The results were 1.7 and 3.3 g ethanol per 100 g corn fiber, respectively for white- and brown-rot fungi, respectively.

Table 1 Specific enzyme activity of crude cell free extract at the beginning of simultaneous saccharification and fermentation of corn fiber into ethanol (adapted from [28]

Enzyme activity tested	White-rot fungus (*P. chrysosporrium)*	Brown-rot fungus (*G. trabeum*)
Alpha amylase (mg maltose/mg protein/min)	0.230 ± 0.06	0.160 ± 0.04
Glucoamylase (mg glucose/mg protein/min)	0.380 ± 0.15	0.180 ± 0.01
Xylanase (mg xylose/mg protein/min)	0.740 ± 0.17	0.060 ± 0.00
Endocellulase (mg glucose/mg protein/min)	0.505 ± 0.05	0.215 ± 0.04
Exocellulase (mg glucose/mg protein/h)	1.030 ± 0.05	0.9063 ± 0.05

5.1 Commercial Enzyme versus Fungal Saccharification

SSF of wet-milled corn fiber with commercial cellulase enzyme (Spezyme CP, 50 filter paper units/g cellulose) had slightly higher ethanol yield compared to solid-substrate cultures with *T. reseei* and subsequent SSF of corn fiber into ethanol. The ethanol yield in the later system was as high as 75% of that obtained in SSF with Spezyme CP system [29]. Whereas the white- and brown-rot saccharification and SSF processes similar to *T. reesei* had lower ethanol yields. Consumption of sugar by fungi was suspected during 4-day long saccharification processes compared to Spezyme-CP coupled SSF process resulting in differential ethanol yields. Combination of fungal saccharification and reduced cellulolytic enzyme (e.g., Spezyme-CP) loading can improve ethanol production from cellulosic substrate.

5.2 Improved Ethanol Yield

As the fungal saccharification process of corn fiber proceeds, fungi also consume released sugar. It is therefore, necessary to minimize the fungal sugar consumption and maximize net ethanol yield. Shorter enzyme induction and fungal saccharification periods and subsequent SSF with *S. cerevisiae* process maximized sequential sugar to ethanol fermentation. To minimize the fungal consumption of sugars, the saccharification process was shortened to 2 days and SSF was incorporated in anaerobic conditions [28]. The enzyme system was active to hydrolyze sugar and sequentially ferment hydrolyzate to ethanol (Table 1). The ethanol yield was as high as 7.8 and 8.6 g ethanol per 100 g corn fiber. Contrary to earlier findings, similar process with *T. reesei* resulted in poor ethanol yield.

6 Economic Implications and Significance

The primary products in corn wet-milling biorefineries are ethanol, starch, high-fructose corn syrup (HFCS), organic acids, corn oil and corn gluten meal. The latter is comprised of basically fibrous residue, i.e. corn fiber (representing bran

coat, germ fiber and tip cap of the corn kernel) that are further blended with gluten (protein) and sold as corn gluten feed and corn gluten meal. Similarly, the dry-grind corn ethanol plants also produce a fibrous co-product: distillers dried grains (DDG) usually combined with solubles and is sold as DDGS. In recent years, the US corn ethanol production (especially from corn dry-grind industries) and percent corn usage for ethanol production has increased exponentially [22, 36]. The amount of co-product such as DDGS has increased in direct proportion to ethanol production. A large amount of energy is spent to produce these co-products but in the long run the supply may exceed demand for this animal feed. High sulfur levels in DDGS also limit ruminant feed supplement to 40–50% [38]. Thus, alternative value-adding processes are required for the profitability of first-generation ethanol plants.

Over 70% of corn fiber is complex carbohydrate (residual starch integrated with hemicellulose and cellulose) on a dry-mass basis. Starch and cellulose fractions of the fiber occur in equal proportions, 18% dry mass each (with some variations), and hemicellulose ($\sim$35%, dry mass) is the highest fraction. The lignin fraction is constitutively very low in corn fiber ($\sim$1.3%, dry mass). Therefore, corn fiber has been primarily sought as a cleaner and valuable feedstock for producing high value products like arabinoxylan gum (from hemicellulose), corn fiber oil, enzymes and ethanol from cellulose and starch fractions. However, the structural complexity and recalcitrance properties of the constituent polysaccharides currently require physico-chemical pretreatment to facilitate enzymatic saccharification. Pretreatment methods are costly and may produce inhibitory compounds. Effective enzyme production for cellulose degradation is still very costly.

Wood-rot fungi (white-rot fungus: *P. chrysosporium* and brown-rot fungus: *G. trabeum*) have been extensively studied for their hemi/cellulose degrading enzymes. The in situ production of these enzymes in solid-substrate or submerged fermentation (under aerobic conditions) was followed by anaerobic SSF with co-culture of *S. cerevisiae* to convert hydrolysate from corn fiber to ethanol. This chapter (i) conceptualizes on-site production of enzymes (from wood-rot fungi), (ii) develops saccharification and fermentation of wet-milled corn fiber to ethanol and (iii) improves the residue quality as an animal feed or development of value-added products by reducing lignocelluloses and increasing protein concentrations.

Studies of amylase, hemicellulase (represented by xylanase) and cellulase (represented by endocellulase and exocellulase) enzyme activities of wood-rot fungi and *T. reesei* showed that the latter fungus has comparable activities on starch, xylan and cellulose. The specific enzyme activities of all three fungi were very high in solid-substrate fermentation compared to submerged fermentation [26, 28]). It is therefore envisaged that starch and hemi/cellulose degrading enzymes production from these fungi can be improved (and concentrated) via solid-substrate fermentation. Solid-substrate fermentation at optimal moisture, temperature and nutrient supply mimics the natural systems of the wood-rot and soft-rot fungi. Therefore, their enzymatic activities are expected to enhance as the fungal growth and metabolism progresses.

Submerged fermentation simplifies controlling pH, temperature and nutrient levels while the fungi grow in the presence of cellulosic feedstock. Fermentors, adequate oxygen supply, intermittent sampling and downstream processing for enzyme harvesting will add extra cost and equipment footprints. Fungal enzyme activities in submerged fermentation will be lower compared to solid-substrate fermentation as observed from the reported experiments due to reduced substrate to fungi intimacy, reduced oxygen concentration compared to air, and dilution of released enzyme into culture medium [18]. It may also stimulate some acetic acid production rather than ethanol (as in case of *T. reesei*) during the SSF process [28]. It is therefore envisaged that commercial solid-substrate fermentation of corn fiber would be more appropriate to produce fungal enzymes using a portion of corn fiber as substrate. The white- and brown-rot fungi had increased enzymatic activities over starch, xylan and carboxymethyl cellulose as compared to corn fiber. This confirms higher amylase, xylanase and endocellulase activities of these fungi. *T. reesei* had higher exocellulase activity compared to wood-rot fungi. All these enzyme activities are necessary to hydrolyze starch, cellulose and hemicellulose fractions of corn fiber into fermentable sugars. The exocellulase activities of *T. reesei* complement the consortia of enzymes secreted by wood-rot fungi. Therefore, a mixed culture of the fungi (white-rot and *T. reesei*; brown-rot and *T. reesei*; white- and brown-rot fungi) can be used for solid-substrate fermentation.

Fungal proliferation would enhance enzymatic activities and may further degrade substrate to provide sugars for fungi. Fungal sugar consumption should be restricted or kept at a minimum. Periodic enzyme harvesting from solid-substrate cultures of fungi followed by purification and concentration would ensure the enzyme supply and quality. This may also help to mitigate the problem of fungal sugar consumption.

The higher the enzymatic activities, the faster and higher would be the ethanol production in the SSF process. The results strongly support hydrolytic fungal enzyme production in solid-substrate fermentation compared to submerged culture. Purified and concentrated enzyme consortia from submerged cultures would definitely improve the corn fiber to ethanol yield in the sugar to ethanol fermentation process. As briefly explained earlier, the SSF of corn fiber preceded by enzyme induction in submerged fermentation using brown- and white-rot fungi had ethanol yields of 42 and 34% of the theoretical maximum yield for C-6 sugars, respectively, (ca. 20.4 g ethanol per 100 g of corn fiber) from an estimated 18% starch and 18% cellulose fraction in corn fiber. These ethanol yields were higher when compared to the ethanol yield from a similar submerged culture SSF study with *T. reesei*, which had 20% of theoretical maximum ethanol yield for C-6 sugars. However, solid-substrate fermentation of corn fiber using these fungi and the sub-sequent SSF process had a higher ethanol yield for *T. reesei* (25% of the theoretical maximum yield). It must also be understood that the fungi would also consume sugar as they proliferate during the enzyme induction phase for solid-substrate fermentation. This would then result in reduced ethanol yields of 25–42% of the theoretical maximum.

7 Process Improvements

The corn fiber to ethanol production trends in the fungal SSF process leveled off after 2 days of fermentation. Extension of anaerobic conditions to 6 or 8 days increased but did not significantly improve the ethanol yield. Therefore, an extended period for SSF fermentation would not be necessary. Ethanol yield had not significantly improved after mild alkali and alkaline peroxide pretreatment of wet-milled corn fiber [29]. Therefore, pretreatment of corn fiber prior to fungal saccharification and fermentation would not be necessary. A separate SSF process for corn fiber would require more reactors, higher retention time for fermentation and additional processing steps. In addition, a separate SSF process for converting corn fiber to ethanol would not be economical in terms of the highest corn fiber to ethanol conversion and concentration of ethanol in the fermentors. At ca. 30% solid loading (corn fiber) the theoretical maximum ethanol concentration would be around 61 g/L (i.e., 6.1% w/v or 7.7% v/v). Such a low ethanol concentration will be at the low end for economical separation by distillation. It would be more practical to add the germ fiber and fiber separated in the upstream process together with corn-starch into the fermentors. The fungal enzymes, collected from solid-substrate fermentation, and commercial amylase enzymes (lesser quantity than required as fungi also have amylase activities) will saccharify the free and residual starch, hemicellulose and cellulose. The fermentable sugar (glucose) is converted into ethanol by *S. cerevisiae*. Conversion of xylose to ethanol is also a possibility. Usually it takes 48–72 h to maximize conversion of sugar to ethanol in ethanol biorefineries. Integration of corn fiber and starch hydrolysis in the same fermentor and conversion of sugar to ethanol would, therefore, be anticipated to yield more ethanol within 48–72 h.

7.1 Usage of Fermentation Residue and Broth

The corn to ethanol conversion rate (gallons of ethanol per bushel of corn) is expected to increase. Utilization of starch and cellulose fraction of corn kernel would reduce bulk generation of solid residue. The residue is also expected to be higher in hemicellulose content (depending on enzyme supplementation) and therefore, be a more favorable feedstock for hemicellulose applications such as the production of arabinoxylan gum. The liquid fraction may be further explored to separate valuable products like metabolites, enzymes, and nutrients. Recycle of enzymes and nutrients back to upstream processes is a possibility. The solid residue can otherwise be supplemented with fungal enzyme complex. The enzyme-supplemented residue could be used as enriched animal feed upon approval after feed trial.

Availability and compositional variability of lignin, hemicellulose and cellulose in various lignocellulose feedstocks like corn stover, bagasse, switchgrass, etc.

greatly determine the desirable end product(s). Higher cellulosic content would favor cellulose ethanol production along with separate end usage of hemicellulose and lignin. Higher lignin content may route the processing towards economical usage of lignin. Unlike corn fiber, lignocellulosic biomass may require mild to severe pretreatment prior to biological conversion of feedstock to sugars and other derivatives. It is also desirable that both physical–chemical and biological pretreatments keep the substrates (and polymers) in their natural states and yet enhance the enzymatic hydrolysis process [2].

Wood-rot and soft-rot fungal treatment of lignocellulosic feedstock can be applicable to untreated or pretreated substrate. Since each and every microbial species has different types and strength of enzymatic activities, in most cases a mixture of microbial population would benefit in holistic degradation of complex polymer to simple sugars, which can further be fermented to ethanol. For example, white-rot and soft-rot fungi (*P. chrysosporium* and *T. reesei*) can be co-cultured in solid-substrate fermentation to provide a complete consortium of lignin and hemi/cellulose degrading enzymes such that the hydrolysate can be completely fermented to ethanol using genetically modified yeasts or bacteria. In many cases, it is also desirable to have multiple products in addition to ethanol. Conversion of glucose and xylose to ethanol and xylitol respectively, may be profitable. Brown-rot fungus (*G. trabeum*) can be used to solely convert lignin and hemicellulose rich feedstock into modified lignin, hemicellulose and cellulose hydrolysate. The lignin and hemicellulose fraction may be used separately for other purposes. There are many possible products other than ethanol from lignocellulose. Contrary to ethanol production, depending on market demand and product values, biomass feedstock can also be utilized for production of organic acids, anti-oxidants, enzyme assisted improved animal feed as conceptualized in Fig. 2.

7.2 Converting Hydrolyzed Sugars to Bio-Oil

Van Leeuwen et al., developed another approach to using the sugars from fungal lignocellulose hydrolysis. They used yet another fungal process to convert the sugars into a fungal oil. This required some mild ammonia pretreatment with full recovery of chemicals as well as lignin as yet another byproduct. The fungal cells, containing the oil are readily separated by simple screening of fungal pellets, and following cell rupture, the oil can be separated and converted into biodiesel. Biodiesel is a better fuel than ethanol and this process represents a better alternative to fungal biofuel production. The 2009 R&D 100 award winning process is shown in Fig. 3. The moist solids from the ammonia pretreatment are subjected to wood-rot fungal fermentation in an aerobic solid-state fermentation (SSF) reactor. The fungus of choice, the white-rot mould–*P. chrysosporium* produces a variety of enzymes, which degrade and saccharify various components of the plant biomass. These sugars are flushed from the biomass with water on a regular daily basis. The hydrolysate is fed into the Mucor reactor, where *M. circinelloides* uses the sugars

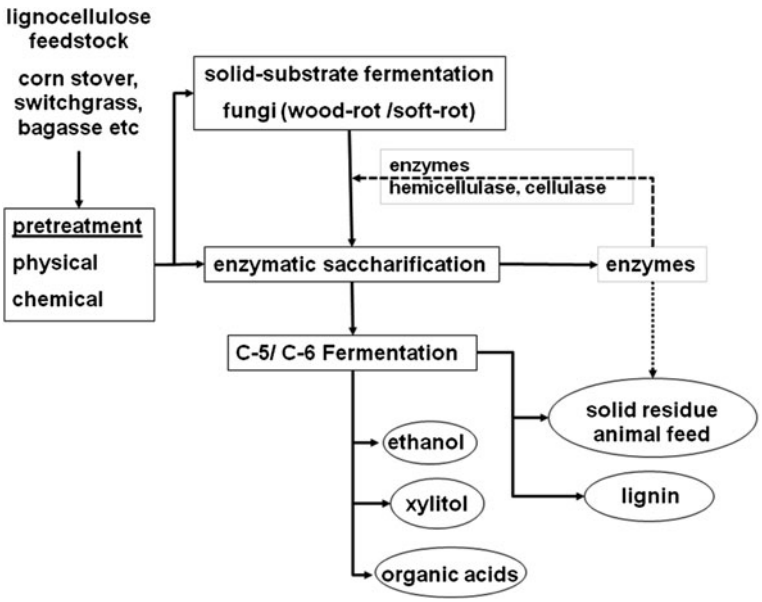

Fig. 2 Integrated biorefinery concept to utilize lignocellulosic biomass for various products like fungal enzymes, alcohols, organic acids, lignin etc. (adapted from [26])

and other nutrients to grow the population and convert excesses to lipids. The solid residues from the SSF reactor can be used as fuel to off-set energy costs or as a soil amendment additive.

8 Summary

The fibrous co-product generated in corn-ethanol industries could serve as suitable cellulosic feedstock to produce second-generation biofuels. Biological pretreatment and enzymatic degradation of lignocellulose into fermentable sugar is an environmental friendly process. Cost of biomass treatment and volumetric enzyme dosage can be reduced via in-house enzyme production by growing fungal cultures on cellulosic substrate such as corn fiber and converting released sugar into ethanol. Conversion of polysaccharide fractions (starch, cellulose, and hemicellulose) from these corn-ethanol co-products into ethanol would yield an additional 25% of current annual ethanol production capacity (i.e., 13×10^9 l of ethanol). This could result in an expansion of $8.65 billion and also increases net ethanol production per bushel of corn or opens the alternative route to convert cellulosic sugars into fungal oil to produce a form of biodiesel.

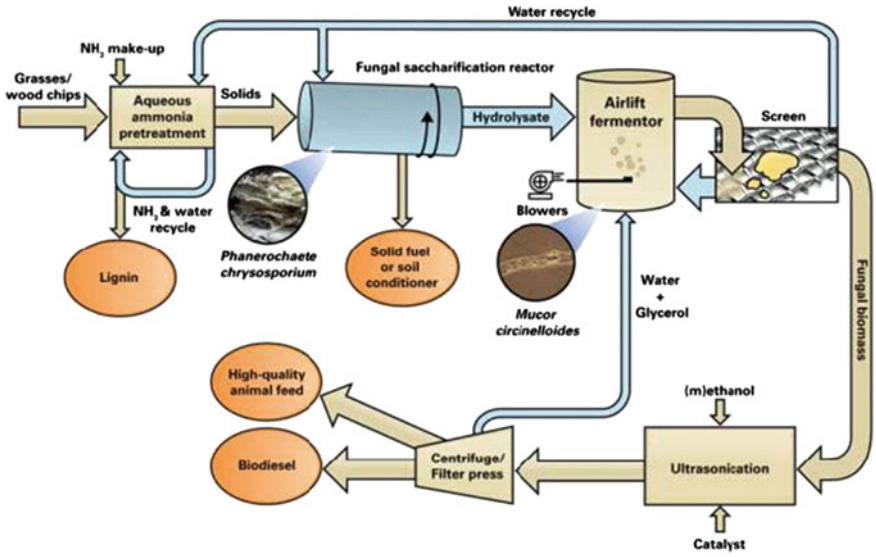

Fig. 3 Integrated Mycofuel fungal process for converting lignocellulosic material into biodiesel ([39] R&D 100 entry, 2009)

References

1. Abbas C, Beery K, Dennison E, Corrington P (2004) Thermochemical treatment, separation and conversion of corn fiber to ethanol. In: Saha BC, Hayashi K (eds) Lignocellulose Biodegradation. American Chemical Society, Washington, pp 84–97
2. Alvira P, Tomas-Pejo E, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 101(13):4851–4861
3. Banerjee G, Scott-Craig JS, Walton JD (2010) Improving enzymes for biomass conversion: a basic research perspective. Bioenergy Res 3:82–92
4. Brown DE (1983) Lignocellulose hydrolysis. Philosophical Transactions of the Royal Society of London, Series B. Biol Sci 300(1100):305–322
5. Chum HL, Douglas LJ, Feinberg DA, Schroeder HA (1985) Evaluation of pretreatments of biomass for enzymatic hydrolysis of cellulose. Technical Report Solar Energy Research Institute, Golden, CO (USA); Colorado State University, Fort Collins (USA)
6. Dale BE (1987) Lignocellulose conversion and the future of fermentation biotechnology. Trends Biotechnol 5(10):287–291
7. Dincer I, Dost S (1997) Energy and GDP. Int J Energy Res 21:153–167
8. Energy Information Administration (EIA). (2010). International energy outlook 2010. Retrieved April 2, 2011 from http://www.eia.doe.gov/oiaf/ieo/world.html
9. Fan LT, Young-Hyun L, Gharpuray MM (1982) The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis. Adv Biochem Eng/Biotechnol 23:157–187 Springer, Berlin
10. Hall DO, Rosillo-Calle F, Williams RH, Woods J (1992) Biomass for Energy: Supply prospects. Renewable Energy–Sources for Fuels and Electricity. Eds Johansson TB, Kelly H, Reddy AKN, Williams RH. Island Press, Washington D.C., 593–652

11. Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD (2007) Biomass recalcitrance: Engineering plants and enzymes for biofuels production. Science 315(5813):804–807
12. Himmelsbach JN, Isci A, Raman DR, Anex RP (2009) Design and testing of a pilot-scale aqueous ammonia soaking biomass pretreatment system. Appl Eng Agric 25(6):953–959
13. Isci A, Himmelsbach J, Pometto A, Raman R, Anex R (2008) Aqueous ammonia soaking of switchgrass followed by simultaneous saccharification and fermentation. Appl Biochem Biotechnol 144(1):69–77
14. Johansson TB, Kelly H, Reddy AKN, Williams RH (1992. Renewable fuels and electricity for a growing world economy–Defining and achieving the potential. Renewable Energy–Sources for Fuels and Electricity. Eds Johansson TB, Kelly H, Reddy AKN, Williams RH. Island Press, Washington D.C., 1–72
15. Millett MA, Baker AJ, Satter LD (1976) Physical and chemical pretreatments for enhancing cellulose saccharification. Biotechnol Bioeng Symp 6:125–153
16. Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Landisch M (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686
17. National Renewable Energy Laboratory–NREL (1993) NREL Getting Extra "Corn Squeezins". Technology Briefs. http://www.nrel.gov/docs/gen/old/5639.pdf
18. Pandey A, Selvakumar P, Soccol CR, Nigam P (1999) Solid state fermentation for the production of industrial enzymes. Curr Sci 77(1):149–162
19. POET (2011) Project Liberty. Retrieved March 23, 2011 from:http://www.poet.com/innovation/cellulosic/projectliberty/index.asp
20. Puri VP (1984) Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. Biotechnol Bioeng 26(10):1219–1222
21. Rasmussen M, Shrestha P, Khanal SK, Pometto AL III, Van Leeuwen J (2010) Sequential saccharification of corn fiber and ethanol production by the brown rot fungus *Gloeophyllum trabeum*. Bioresour Technol 101:3526–3533
22. Renewable Fuels Association (2011) Retrieved March 23, 2011 from http://www.ethanolrfa.org/industry/statistics/
23. Rosen MA (2002) Energy efficiency and sustainable development. Int J Glob Energy Issues 17(1–2):23–34
24. Saha BC, Dien BS, Bothast RJ (1998) Fuel ethanol production from corn fiber current status and technical prospects. Appl Biochem Biotechnol 70–72(1):115–125
25. Shiu A, Lam PL (2004) Electricity consumption and economic growth in China. Energy Policy 32(1):47–54
26. Shrestha P, Rasmussen ML, Khanal SK, Pometto AL, Van Leeuwen J (2008) Saccharification of corn fiber by Phanerochaete chrysosporium in solid-substrate fermentation and subsequent fermentation of hydrolyzate into ethanol. J Agric Food Chem 56:3918–3924
27. Shrestha P (2008) Enhanced bioprocessing of lignocellulose: Wood-rot fungal saccharification and fermentation of corn fiber to ethanol. Doctoral Thesis. Iowa State University, Ames
28. Shrestha P, Khanal SK, Pometto AL, Van Leeuwen J (2009) Enzyme production by wood-rot and soft-rot fungi cultivated on corn fiber followed by hydrolyzate fermentation to ethanol. J Agric Food Chem 57:4156–4161
29. Shrestha P, Khanal SK, Pometto AL, Van Leeuwen J (2010) Ethanol production via in situ fungal saccharification and fermentation of mild alkali and steam pretreated corn fiber. Bioresour Technol 101(22):8698–8705
30. Singh A, Kumar PKR, Schugerl K (1992) Bioconversion of cellulosic materials to ethanol by filamentous fungi. Adv Biochem Eng /Biotechnol 45:29–55
31. Singh V, Moreau RA, Doner LW, Eckhoff SR, Hicks KB (1999) Recovery of fiber in the corn dry-grind ethanol process: a feedstock for valuable coproducts. Cereal Chem 76(6):868–872
32. So KS, Brown RC (1999) Economic analysis of selected lignocellulose to ethanol conversion technologies. Appl Biochem Biotechnol 79(1–3):633–640

33. South CR, Hogsett DA, Lynd LR (1993) Continuous fermentation of cellulosic biomass to ethanol. Appl Biochem Biotechnol 39–40(1):587–600
34. United Nations Development Programme (UNDP). (2000). World energy assessment. New York
35. United States Department of Agriculture (USDA) and United States Department of Energy (USDOE) (2005) A billion-ton feedstock supply for a bioenergy and bioproducts industry: the technical feasibility of a billion-ton annual supply
36. United States Department of Agriculture (USDA). 2009. USDA AMS Ethanol and CoProducts Reports. http://www.ers.usda.gov/Briefing/Baseline/present2009.htm#cropsbox1
37. United States Energy Information Administration (USEIA) (2011) Annual Energy Outlook. AEO2011 Early Release Overview. Retrieved April 2, 2011 from: http://www.eia.gov/forecasts/aeo/pdf/0383er(2011).pdf
38. Uwituze S, Parsons GL, Schneider CJ, Karges KK, Gibson ML, Hollis LC, Higgins JJ, Drouillard JS (2011) Evaluation of sulfur content of dried distillers grains with solubles in finishing diets based on steam-flaked corn or dry-rolled corn. J Anim Sci, March 2011. Electronic version ahead of printing
39. Van Leeuwen J, (Hans) Kim TH, Grewell D, Mitra D, Beattie S, Ziel C, Chand P, Reddy Chintareddy V, Montalbo-Lomboy M, Verkade J (2009) An Integrated Fungal Biodiesel Production Process: Mycofuel, winning entry for the R&D 100 awards
40. Wiegel J (1982) Ethanol from cellulose. Cell Mol Life Sci 38(2):151–156
41. Yang B, Wyman CE (2008) Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels Bioprod Bioref 2:26–40

Bio-Renewable Asphalt Modifiers and Asphalt Substitutes

Joana Peralta, Mohamed Abdel Raouf, Sheng Tang and R. Christopher Williams

Abstract The global asphalt market is to reach 118.4 million metric tons by 2015, according to a January 2011 report by Global Industry Analysis, Inc. The asphalt paving industry accounts for the largest end-use market segment of asphalt. With increasing growth in the developing markets of China, India, Brazil, and Eastern Europe, asphalt will be needed to construct their roadway infrastructure well into the next decade. The increased demand for asphalt, along with the need for improved asphalt materials/pavement performance, creates the opportunity for bio-renewable asphalt modifiers and/or asphalt substitutes.

1 Introduction

Materials can be classified as natural or synthetic, recycled or virgin, renewable or non-renewable, degradable, non-degradable, or biodegradable. There are no clear-cut answers on which is less environmentally damaging. However, new technologies and incorporation of appropriate advanced technology into product and processes should be safer, more effective, less costly, and furthermore can make a significant contribution to improve their impact on the environment [1]. Sustainability, industrial ecology, eco-efficiency, and green chemistry are guiding the development of the next generation of materials, products, and processes [2].

J. Peralta · M. A. Raouf · S. Tang · R. C. Williams (✉)
Civil Engineering, Institute for Transportation,
Iowa State University, Ames, IA, USA
e-mail: rwilliam@iastate.edu

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*,
Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_6,

Simply stated, bio-based materials include industrial products, not food or feed, made from renewable agricultural and forestry feed stocks, including wood, wood wastes and residues, grasses, crops, and co-products of crops [2].

Most bituminous adhesives or binders that are used for pavement materials are derived primarily from fossil fuels. With petroleum oil reserves becoming depleted and the drive to establish a bio-based economy, there is a push to produce binders from alternative sources, particularly from bio-renewable resources.

Bio-fuels have many advantages over fossil fuels as they are renewable, environmentally friendly, provide energy security, and present a great economic opportunity for the United States. However, until now, almost no research has studied the applicability of utilizing bio-oils as a partial or full replacement alternative of bitumen in the pavement industry. The limited number of references produced on this matter show that this goal may be achieved very soon by the application of a new technology with the aid of polymer addition.

The term bio-polymers correctly imply the use of bio-based materials to produce polymers. This is a very important field of investigation, since polymers as well as asphalt are very much dependent upon the supply of crude petroleum and petroleum production. Additionally, some polymers may be damaging to the environment.

2 Bio-Binders

The United States has numerous efforts underway working to establish a bio-based economy which generates energy from renewable organic matter rather than fossil fuels [3]. Biofuels have many advantages over fossil fuels as they are renewable, environmentally friendly, provide energy security, and present an economic opportunity for the United States [3]. Bio-fuels can be classified as liquid or gaseous fuels and they are produced from plant matter and residues, such as agricultural crops, municipal wastes and agricultural, and forestry by products [3, 4].

2.1 Definition

Bio-binder is an asphalt binder alternative made from non-petroleum-based renewable resources, which should not rival any food material, and have environmental and economical benefits. Presently, bio-binders are produced by upgrading bio-oils produced from the rapid heating of biomass in a vacuum condition.

By definition, bio-oils can be described as dark brown, free-flowing organic liquids that are comprised mainly of highly oxygenated compounds [4, 5]. In other words, it is the liquid produced from the rapid heating of biomass in a vacuum condition [6]. Bio-oils have many synonyms that can be listed as follows: pyrolysis oil, pyrolysis liquid, bio-crude oil, liquid wood, wood oil, liquid smoke, wood distillates, and pyroligneous acid [4, 6].

Due to the variety of forestry and agricultural sources from which bio-oils are derived, bio-oils are a complex chemical mixture of water, guaiacols, catecols, syringols, vanillins, furancarboxaldehydes, isoeugenol, pyrones, acetic acid, formic acid, and other carboxylic acids [4]. Also, bio-oils encompass other major groups of compounds, including hydroxyaldehydes, hydroxyketones, sugars, carboxylic acids, and phenolics as reported by [4]. As a result of the presence of cellulose, hemicellulose, and lignin in forestry and agricultural crops, the production of bio-oils can be described as the rapid and simultaneous depolymerization and fragmentation of these compounds while rapidly increasing the temperature [4].

According to a literature review conducted by [4], there are many unusual attributes for the bio-oils because of the complexity and the redundancy of the chemical structure of the bio-oils. Even though the recovery of pure compounds from the complex bio-oils is technically and chemically feasible, it is not economical due to costs for recovery of the chemical and its low concentration in the oil as claimed by [3].

Bio-oils are derived from biomass which contains oligomeric species that consist mainly not only of lignin, but also from cellulose and hemicellulose. As decomposition rapidly occurs, oligomeric species are not vaporized but simply blown apart into aerosols. Thus, these oligomeric species form as part of the aerosols and have various molecular weights [4].

2.2 Primary Sources of Biomass Materials

Over the years, biorenewable natural resources including sugars, triglyceride oils, and proteins have been tested as alternative sources for producing adhesives and binders [7]. For example, adhesives derived from soy protein, starch, cellulose, and other polysaccharides have been extensively used for adherents such as wood, paper, plastic, metal, leather, and glass [7, 8]. Due to the availability of large quantities of biorenewable sources such as triglyceride oils, proteins, starch, and other carbohydrates from different botanical sources, there are virtuous technical and economic prospects in utilizing them to produce bio-binders [7]. Recently, through the application of scientific research and development, a range of different vegetable oils have been investigated to determine their physical and chemical properties to study their applicability to be used as bio-binders in the pavement industry [7, 9, 10].

Bio-oils are produced from plant matter and residues, such as agricultural crops, municipal wastes, and agricultural and forestry byproducts [3, 4]. Other biomass sources include sugar, molasses and rice, corn and potato starches, natural tree and gum resins, natural latex rubber and vegetable oils, lignin, cellulose, palm oil waste, coconut waste, peanut oil waste, canola oil waste, potato starch, dried sewerage effluent, and others.

In the plant kingdom, lignin is present in large amounts in the cell wall of plants, especially in woody tissues. It is known that lignin can have different chemical composition and properties from trees, plants, and agricultural crops. Even by operating different extraction methods, the physical and chemical properties are diverse [11]. Since the scavenging ability of lignin depends on the structural features of the lignin, the same amount of lignin with different structures may react with different amounts of free radicals [11]. That is to say, the bio-oil with higher amounts of lignin does not have to display better antioxidant effects.

Presently, a new technology is being applied to produce bio-binders using oak wood, switch grass and corn stover. The bio-binders produced with these materials presents properties similar to those of the conventional asphalt binders.

2.3 Fields of Application

Generally, fast pyrolysis does not generate any waste because the bio-oil and solid char can each be used as a fuel and the gas can be recycled back into the process [4]. According to Goyal et al. [12], the bio-oils obtained from pyrolysis methods have many industrial uses that include but are not limited to use as a combustion fuel, a transportation fuel to substitute fossil fuels, a liquid smoke, a preservative, a raw material to produce chemicals and resins, a binder for pelletizing and briquetting of combustible organic waste materials, or an adhesive material that can be used as a binder in the paving industry. In addition, the char can be utilized in many industrial usages including use as a solid fuel in boilers, as briquettes that are mixed with biomass to be used as high efficiency fuel in boilers, as a raw material to produce activated carbon or carbon nanotubes, or in the gasification process to obtain hydrogen rich gas by thermal cracking [12]. Furthermore, pyrolysis gases which have significant amount of carbon dioxide along with methane can be used as a fuel for industrial combustion purposes [12].

The main field of application of bio-binders is in the paving industry; however, market opportunities exist in housing products via shingles and sealants. Bio-binders can be utilized in three different ways to decrease the demand for fossil-fuel-based bituminous binders: a direct alternative binder (100% replacement), a bitumen extender (25–75% bitumen replacement), or a bitumen modifier (< 10% bitumen replacement).

Williams et al. [13] conducted research on the usage of bio-oils fractions as an extender in original and polymer-modified asphalt binders. They reported that the bio-oils can considerably increase the performance grade of polymer-modified asphalt binders by nearly 6°C [13]. In addition, it was concluded that the effect of bio-oils was dependent upon many factors including the base asphalt, source of the biomass from which the bio-oils were derived, and the percentage of bio-oils blended with asphalt binders [13]. Moreover, Williams et al. reported that up to 9% of a bio-oil could be blended with asphalt binders with significant improvement in performance grade of the bio-oil-modified asphalt binder.

In asphalt or flexible pavements, all types of failure or distress can be classified by whether they are structural or functional failures and load-associated or non-load-associated distresses. Surface course aging is considered as a non-load-associated distress caused by climatic/environmental effects. Many environmental factors can cause surface course aging damage, such as ozone, ultraviolet rays, oxygen, and thermal radiation. Oxidative aging causes the asphalt binder to become harder and more brittle and thus more susceptible to thermal cracking [14].

The oxidation of asphalt binder caused by chemical reactions makes some transformations between the asphalt components. Asphalt oxidation is the main cause of long-term deterioration and eventually results in thermal cracking in asphalt pavements [15−17]. The oxidation causes a substantial increase in the asphaltenes with a higher molecular weight and is correlated with asphalt hardening.

The common asphalt additives such as polymer, rubber, and plastic do not prevent oxidative aging of the binder. The polymer additives can react with the free radicals and then the degradation happens to the additives. The polymer and other rubbers cannot prevent the propagation of the free radical or peroxy radicals because the polymer degradation can form new free radicals and the cycle repeats [18].

Lignin contains several functional chemical groups, such as phenolic, methoxyl, carbonyl, and carboxyl. Most antioxidant effects of lignin are provided by the phenolic group capturing and reacting with the free radicals which contain oxygen. Free radicals are known to "attack" stable molecule structures of a substance by "stealing" an electron. Once the "attacked" molecule loses its electron, the substance's chemical structure is then changed. When the chemical reaction occurs, new materials are formed and the asphalt starts aging with asphaltenes generated. Instead of letting the free radicals, which contain the oxygen, oxidize the aromatics and convert aromatic molecules to asphaltenes, the lignin first reacts with those free radicals as a reducing agent. This radical scavenging property of lignin makes it recognized as an efficient antioxidant additive.

2.4 Importance of This New Binders, Impact on the Paving Industry

Research has been done by adding various chemicals as antioxidant additives. No additive has been successful due to the environmental and economic concerns. Lead diamyldithiocarbamate does have an antioxidant effect, but it contains lead and is not an environmentally friendly material. Zinc kailkyldiathiophashate and zinc diabutyldithiocarbamate have been tested and proven to have antioxidant effects in asphalt binder [18], but their application can be limited because of the relatively high costs.

The bio-oil generated from waste bio-mass at low cost is an environmentally friendly material containing the natural antioxidant lignin. The bio-oil shows

potential to be successfully applied as an antioxidant additive in asphalt pavements.

2.5 Overview of the Bio-Binders Production Technology

Bio-fuel production plants produce liquid co-products that are high in lignin content. Due to that, bio-oils have been used in many traditional uses which include but are not limited to concrete admixtures, binders, well drilling mud, dust control, vanillin production, and dispersants [13]. Lignin, which is a biological polymer, is known as an antioxidant compound due to the presence of large amounts of phenolic structures. Due to its dark color, lignin has not been utilized in many industries; however, this is not an issue when it is used in asphalt binders [13]. Due to the results of some investigations, it has been found that lignin can be utilized as an extender in asphalt to help reduce the use of petroleum with no adverse effects on performance [13, 19, 20].

Currently, the state of the art for the utilization of bio-oils is concentrated on its uses as biorenewable fuels to replace fossil fuels. However, there has been a limited amount of research conducted to investigate the applicability of using bio-oils as a bitumen modifier or extender. Based on the conclusion of these investigations, the utilization of bio-oils as a bitumen modifier is very promising. On the other hand, there has been no research conducted until recently that studies the applicability of the utilization of bio-oils as a bitumen replacement (100% replacement) to be used in the pavement industry. As a result, there is scarcity of data that illustrate the procedure to develop bio-binders from bio-oils.

Biomass, which are most often agricultural and forestry residues, contains a significant amount of carbohydrates, e.g. cellulose, hemicelluloses, and lignin. Therefore, bio-fuels are produced from biomass through biochemical or thermochemical processes. In general, carbohydrates are potential sources for the production of bio-fuels and chemicals [21]. By hydrolysis processes, carbohydrates can be converted to sugars and then subsequently through fermentation, such as an anaerobic biological process; sugars are converted to biofuels by the action of microorganisms, usually yeast [21]. Depending upon the process of converting plant matter to bio-fuels, different co-products are produced. Some of these co-products are not utilized in any other industry; therefore, more effort should be placed on discovering new uses and applications for these co-products. Utilizing the co-products is crucial for the success and profitability of the whole bio-fuels production industry [22, 23].

One of the thermochemical processes used to produce bio-oils is fast pyrolysis. The yields from fast pyrolysis are varied with the biomass feed stock and the reactor conditions [24]. Generally, this process generates bio-oil, bio char, and some gas and moisture (Fig. 1). The bio char can be used for carbon sequestration as a soil modifier by improving the soil's ability to retain liquid fertilizers and thus reducing liquid fertilizer application rates. The bio-oil is a liquid fuel containing

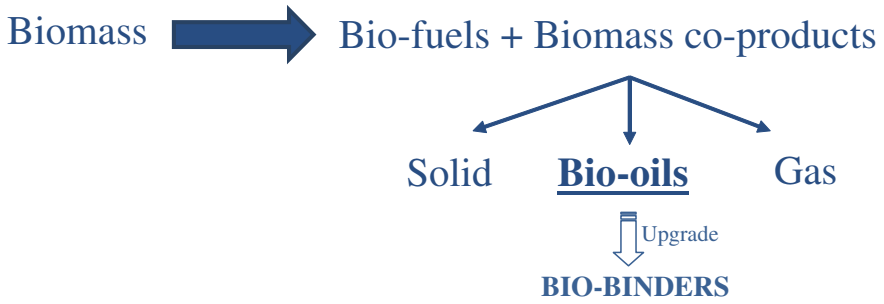

Fig. 1 Production of bio-fuels, bio-binders, and other co-products by the fast-pyrolysis thermochemical process

lignin that can be combusted by some engines or turbines for the electricity generation purpose [25]. Since, bio-oils cannot be used as bio-binders/pavement materials without any heat pre-treatment, an upgrading procedure is required.

Fast pyrolysis is a thermal decomposition process that requires a high heat transfer rate to the biomass particles and a short vapor residence time in the reaction zone [5]. In other words, fast pyrolysis is the rapid decomposition of organic matter (biomass) in the absence of oxygen to produce solids as char, pyrolysis liquid or oil (bio-oils), and gas [21, 26]. Another detailed definition of fast pyrolysis is given by Mohan et al. [4] which describes fast pyrolysis as a high-temperature process in which biomass is rapidly heated in vacuum and then decomposes to produce vapors, aerosols, and some charcoal-like char and after cooling and condensation of these vapors and aerosols, a dark brown mobile liquid (bio-oils) is formed.

When the organic matter is biomass, the produced oils are named bio-oils. Generally, fast pyrolysis is used to obtain high-grade bio-oil. Organic biomass consists of biopolymers, e.g., cellulose, hemicelluloses, and lignin. Therefore, fast pyrolysis of lignocellulosic biomass leads to extensive depolymerization and fragmentation of these biopolymers [26]. Due to the different sources of biomass, the amount of production of the liquid bio-oils, solid char, and non-condensable gases vary. For example, fast pyrolysis processes produce about 60–75 wt% of liquid bio-oil, 15–25 wt% of solid char, and 10–20 wt% of non-condensable gases [4].

Figure 2 shows the 25 kWt fast-pyrolysis system developed at Iowa State University by the Center for sustainable Environmental Technology where bio-oils extracted from different biomass materials are produced.

The pilot unit consists of a 16.2 cm diameter fluidized bed reactor, a burner to externally heat the reactor, a two-stage auger to feed the solid, two cyclones to remove particulate matter, and a vapor-condensing system consisting of four condensers, and an electrostatic precipitator. The system can process 6–10 kg/h of solid feed. The separation of bio-oils into multiple fractions was conducted using a fractionation condenser system which facilitated the selection of bio-oil fractions that would be optimal for being used as a pavement binder.

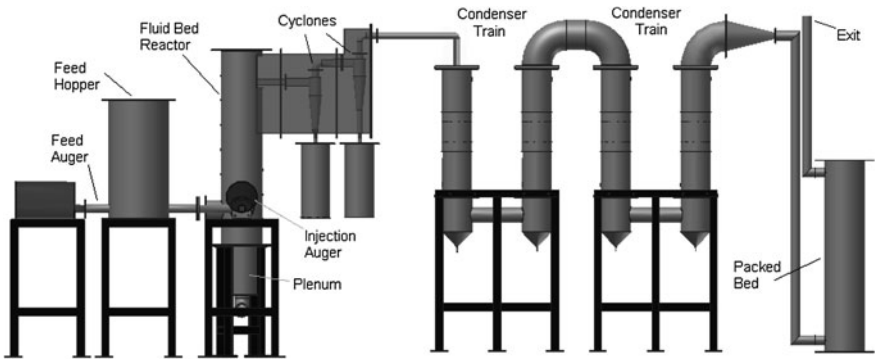

Fig. 2 The Bio-oil mass pyrolysis pilot plant (Source: Iowa State University)

Some researchers reported that bio-oils produced from fast pyrolysis have some potential problems. Mullen and Boateng [26] reported that bio-oils have high oxygen and water content which leads to poor volatility, high viscosity, and corrosiveness. In addition, bio-oils have hundreds of various oxygenated organic compounds that are highly reactive leading to instability problems and an increase in viscosity over time [26].

Mohan et al. [4] reported that almost 200 intermediate products formed during the pyrolysis of biomass and cellulose is the major constituent. Despite that wide variety of products, most of these products, such as bio-oil, solid char, and gases can be re-used in different ways. The amount and distribution of the solid, liquid, and gas formed during the pyrolysis depends on the process variables, such as type of biomass and catalytic process and temperature [4].

It initially starts with slow heating rates, and then involves a rapid heating rate of biomass, that can reach up to 300°C/min, but not as fast as flash pyrolysis. According to [12] and Luo et al. [27], fast pyrolysis is most successful with fluidized bed reactors as it offers high heating rates, rapid de-volatilization, easy control, and easy product collection. Fast-pyrolysis design variables include but are not limited to the following: feed moisture content, particle size, pretreatment, reactor configuration, heat supply, heat transfer, heating rates, reaction temperature, vapor residence time, secondary cracking, char separation, ash separation, and liquid collection as reported by [4].

2.6 How are These Materials Characterized and Applied

These conclusions are limited for oakwood, switchgrass, and cornstover bio-oils obtained by fast pyrolysis. The developed bio-binders cannot be treated with temperatures higher than 120°C due to the high oxygen content which will lead to a considerable amount of oxidation with higher temperatures.

Since there are considerable differences between the properties of the bio-oils and asphalt at the same temperatures, the Superpave test criterion should be modified to comply with the bio-binders properties, namely, the Superpave specifications for the rolling thin film oven test (RTFOT) and the pressure aging vessel procedures.

Based on the tested bio-oils, the following suggestions were made. First, the RTFOT temperature should be modified to 110–120°C instead of 163°C to be consistent with the intended mixture production temperature. Also, the 20-min duration was established to be the duration to resemble the mixing and compaction duration. Second, the aging duration in the PAV oven should be shortened to 2.5 h instead of 20 h and the temperature of the degassing container should be lowered to 120°C instead of 170°C.

For the physical testing, the following observations were noted. First, the oakwood bio-binders generally were more susceptible to separation with polymer modifiers used in this research in comparison to switchgrass and cornstover bio-binders. Therefore, more care and caution should be taken when blending oakwood bio-binders with polymer modifiers. Second, the specific gravity values of the bio-binders were higher than the specific gravity values of the bitumen binders. Third, there was no significant difference between the specific gravity values of the unmodified bio-binders (oakwood, switchgrass, and cornstover).

For the chemical testing, the following remarks are made. First, it was observed that the furfural and the phenol compounds might be reacting with each other and forming a new polymer due to the heat treatment/upgrading procedure and the aging processes; however, the phenol compounds, which are acting as an antioxidant agent, are still present, unlike the furfural compounds which were completely removed, after the heat pre-treatment/upgrading procedure and the aging processes. Second, the aging ratios for both reacting groups (CH2 and CH2–CH3) with respect to the neutral O–H group were decreasing which meant that these reacting groups were decreasing upon heat pre-treatment/upgrading procedure and aging processes. Third, for the aging indexes, upon heat pre-treatment/upgrading procedure and aging processes of the unmodified bio-binders, the aging indexes were generally increasing but without a specific trend. Importantly, these two new means or methods, i.e., aging ratio and aging index, can be employed to quantify the amount of aging occurring on some of the bio-binders, such as oakwood and switchgrass, upon heat treatment and aging processes, but care should be taken before usage of these means or methods as their validity vary depending on the type of the bio-binders.

For the rheological testing, the overall conclusions, which included shear and temperature susceptibilities, behavior index n, consistency index K, and activation energy Ea can be summarized as follows. First for the shear susceptibility (SS), although the SS values of the switchgrass and cornstover bio-binders yielded higher values than the bitumen binders tested, the statistical analysis showed that there was no statistical difference. In addition, the addition of polymer modifiers with different blending ratios did not lead to significant changes in the SS values of all types of the bio-binders. However, the polymer modifiers changed the

temperature range of the oakwood and switchgrass bio-binders only and had no effect on the cornstover bio-binders. Moreover, the addition of different types of polymer modifiers was not yielding the same effect on the different types of bio-binders. Importantly, the relationship between viscosity and shear rate at different temperatures, for different types of bio-binders, can be well described by a similar linear logarithmic relationship as bitumen binders. Second, for the temperature susceptibility, it can be concluded that the temperature was the main contributor to the viscosity of the bio-oils in comparison to shear rate. In other words, the effect of temperature in changing the viscosity of the bio-oils was more significant than the effect of shear rate. This kind of behavior showed that the bio-oil binders had the same behavior as bitumen binders. In addition, the relationship between viscosity and temperature at different shear rates can be well described as a linear logarithmic relationship. Importantly, it was observed that the addition of polymer modifiers with different blending ratios did not lead to the same effect when blending with different bio-binders. Also, the effect of the addition of different types of polymer modifiers and the blending ratios on the viscosity temperature susceptibility (VTS) values varied depending on the type of the bio-binder and the shear rate. Third, for the behavior index n, it can be concluded that increasing the temperature led to a more Newtonian behavior for the oakwood and switchgrass bio-binders (n values were almost equal to unity), but the cornstover bio-binders were not following the same behavior. Also, all the bio-binders at low temperatures had a pseudo-plastic behavior as their n values were less than unity.

Fourth, for the consistency index K, it was observed that increasing the temperature led to a decrease in the viscous behavior of all types of bio-binders. Based on the statistical analysis conducted, it may be concluded that the addition of polymer modifiers with different blending percentages to all types of bio-binders did not generally lead to a significant change in the n and K values. Significantly, there was no statistically significant difference between n and K values of the unmodified bio-binders and bitumen. In addition, there was no significant difference between n and K values of modified oakwood bio-binders and the bitumens. On the other hand, there was no significant difference between n values of modified switchgrass and the bitumens, but there was significant difference between K values of modified switchgrass and bitumen. For the cornstover bio-binders, for the n values, there was a significant difference between the modified cornstover bio-binders and the bitumens, but there was no significant difference between K values of the modified cornstover bio-binders and the bitumens. Therefore, it is worth noting that the effect of the polymer modifiers on the n and K values vary according to the bio-binder type. Considerably, the relationship between viscosity and shear rate at different temperatures for the bio-binders and bitumen were following a power-law relationship.

Fifth, for the activation energy Ea, which represents the susceptibility of bio-binders to temperature, the following observations can be noted: (i) the temperature susceptibility of the unmodified oakwood bio-binders was higher than the temperature susceptibility of the bitumen binders, but there was no significant difference between temperature susceptibility of the modified

oakwood bio-binders and the bitumen binders. However, for the switchgrass and cornstover bio-binders, the temperature susceptibility of the unmodified and modified switchgrass and cornstover bio-binders was not statistically different than the temperature susceptibility of the bitumen binders; (ii) neither polymer type nor the blending ratios affected the *Ea* values of the oakwood, switchgrass, and cornstover bio-binders; (iii) the relationship between viscosity and temperature at different shear rates was well described by the Arrhenius-type model.

For the performance grade testing, the following findings were established based on the performance grade and the mixing and compaction temperatures. First, it is more feasible and reliable to determine the high-temperature performance grade of the bio-binders through the G*/sin(delta) of the RTFO aged samples. Precisely, in this study, the high-temperature performance grades for bio-binders (unmodified and modified) were determined using 20-min RTFO aging residues at 115°C. Generally, it is worth noting that bio-binders can yield the same or even a greater high-temperature performance grade in comparison to the bitumen binders. Second, for the intermediate temperature performance grade, it can be concluded that the unmodified and modified bio-binders had generally higher grades than the bitumen intermediate temperature performance grade. Third, it is worth noting that due to the high oxygen content in bio-binders, the low-temperature performance grade of the tested bio-binders were higher than the low-temperature performance grade of the commonly bitumen binders used in the US market. No clear trend could be observed for the effect of the addition of polymer modifiers in changing the intermediate and low-temperature performance grade for bio-binders. Importantly, it was established that the tested bio-binders should be limited in their use in cold climate regions until their low temperature properties are improved. Fourth, the results revealed that the mixing and compaction temperatures for the bio-binders generally were lower than the typical mixing and compaction temperatures for the bitumen binders commonly used.

For developing master curves for bio-oils, the following conclusions can be made. First, the behavior of the bio-binders (unmodified and polymer modified) varied with respect to their type; therefore, their behavior generally may be different from bitumen. Second, the bio-binders had higher complex moduli at low temperature/high reduced frequency compared to the corresponding values of bitumen. This means that the resistance of bio-binders to thermal cracking should be a main concern in utilizing bio-binders as pavement materials in cold climates. Third, the addition of different types of polymer modifiers with different percentages leads to a change in the shape/trend of the master curve and hence changes the behavior of the bio-binder.

Consequently, before utilizing the bio-binders in pavement applications, different types of polymer modifiers with different percentages should be tested until the required/specified behavior is achieved. Overall, the master curves for different types of bio-binders can be well constructed and predicted using Richard‘s curve.

2.7 Comparison Between This New Material and the Conventional Ones

The temperature range of the viscous behavior for bio-oils may be lower than that of bitumen binders by about 30–40°C. The rheological properties of the unmodified bio-binders vary in comparison to bitumen binders, but the rheological properties of these modified bio-binders change significantly upon adding polymer modifiers. The high-temperature performance grade for the developed bio-binders may not vary significantly from that of the bitumen binders, but the low-temperature performance grade may vary significantly.

2.7.1 Constitution

The physical properties showed that the oakwood bio-binders generally were more susceptible to separation with all types of the polymer modifiers used in this research in comparison to switchgrass and cornstover bio-binders. Therefore, more care and caution should be taken when blending oakwood bio-binders with polymer modifiers. Second, the specific gravity values of the bio-binders were higher than the specific gravity values of the bitumen binders. The chemical properties showed that the amount of furfural and phenols were changing due to the different aging processes and durations. Importantly, two new means and methods were employed to quantify the amount of aging in the unmodified bio-binders; however, the results showed that care should be taken before usage of these means or methods as their validity may vary depending on the type of the bio-binders.

Results reveal that the relationship between the viscosity of bio-oils and temperature and shear rates are log linear-like bitumen binders. In addition, temperature is the main contributor to the viscosity of the bio-oils in comparison with shear rate. Important is that the viscosity temperature susceptibility values for the bio-oils in comparison with bitumen blends indicate that bio-oils are more susceptible to temperature. Moreover, the addition of polymer modifiers leads to a change in temperature ranges of the bio-oils. In conclusion, the rheological properties of oakwood bio-oils are similar to and comparable with bitumen binders, and they represent a viable renewable alternative to petroleum-derived asphalt binders.

Similar to asphalt, the chemistry of bio-oils is complex [4]; thus, a complete chemical characterization is difficult or almost impossible. The complexity of chemical characterization or analysis resulted from the presence of high molecular weight of phenolic species from lignin decomposition [4]. In addition, the fragmented oligomeric products exist with different numbers of phenolic and carboxylic acids, and hydroxyl groups as well as aldehyde, alcohol, and ether functions. Thus, phenolic species exist as different hydrogen-bonded aggregates, micelles, droplets, and gels.

2.7.2 Performance

Current testing standards and specifications, especially Superpave procedures, should be modified to comply with the properties of the bio-binders derived from bio-oils as the chemical composition is substantially different than those of petroleum-derived asphalt which leads to differences in temperature susceptibility and aging of the two materials.

The temperature range of the viscous behavior for bio-oils may be lower than that of bitumen binders by about 30–40°C. The rheological properties, i.e., temperature and shear susceptibilities, of the unmodified bio-binders derived from bio-oils vary in comparison to bitumen binders, but upon adding polymer modifiers, the rheological properties of these modified bio-binders change significantly.

The high-temperature performance grade for the developed bio-binders may not vary significantly from the bitumen binders; however, the low-temperature performance grade may vary significantly due to the high oxygen content in the bio-binders and subsequent aging compared to the bitumen binders.

The applicability of using bio-oils as bio-binders in the pavement industry can be summarized as follows:

- The bio-oils cannot be used as bio-binders/pavement materials without any heat pre-treatment/upgrading procedure due to the presence of water and volatile contents in considerable amounts.
- The heat treatment/upgrading procedure for deriving bio-binders from bio-oils should be determined for each type of bio-oil separately. This is mainly due to the significant difference between the different types of bio-oils. For example, the chemical composition of the different types of bio-oils vary significantly based on many factors, e.g., the process by which the bio-oils are produced and the type of the biorenewable resource from which the bio-oils were derived.
- The current testing standards and specifications, especially Superpave specifications, should be modified to comply with the properties of the bio-binders derived from bio-oils. This is mainly due to the difference in the chemical structure and composition between bio-oils and crude-petroleum binders. Importantly, due to the considerable amount of oxygen in the bio-oils, new specifications and testing procedures should be developed for the bio-binders derived from bio-oils.
- The temperature range of the viscous behavior for the bio-oils should be determined precisely as the temperature ranges for the different bio-binders vary depending on the type of the bio-oil and the type of the polymer modifier used. In general, the mixing and compaction temperature range for bio-oils may be lower than that of bitumen binders by about 30–40°C.
- The rheological properties, i.e., temperature and shear susceptibilities, of the unmodified bio-binders derived from bio-oils vary in comparison to bitumen binders, but upon adding polymer modifiers, the rheological properties of these modified bio-binders change significantly.

- The polymer modifiers should be chosen with care and caution because the temperature range of the developed bio-binders is different than the polymer modifiers commonly used in the bitumen industry.
- The high-temperature performance grade for the developed bio-binders may not vary significantly from the bitumen binders. However, the low temperature performance grade may vary significantly due to the high oxygen content in the bio-binders compared to typical bitumen binders.

2.8 Can This Material Substitute the Conventional Petroleum Asphalt in the Near Future?

There are still some technological aspects to improve, but according to actual state of the art, this technology will be available or field application in pavements soon. However, this effective application may depend more on economical issues than technological ones.

Extensive testing has brought to light many issues that are involved in the utilization of bio-oils as bio-binders in the pavement industry. The recommendations for future work can be summarized as follows:

- More investigation is required to study the applicability of using the bio-oils as bio-binders through studying and testing other bio-oils derived from different sources of biomasses.
- The effect of different types of polymer modifiers on the different types of bio-oils should be studied extensively. Particularly, the effect of molecular weight of the polymer modifiers on the rheological properties of the bio-binders.
- The thermal expansion coefficient of the developed bio-binders may be investigated particularly at low temperatures.
- More research effort should be conducted to study the applicability of using bio-oils as a direct alternative binder (100% replacement) in the pavement industry including mix designs and subsequent performance testing of mixes containing bio-binders.
- Additional investigation is needed to validate the heat pre-treatment/upgrading procedure recommended by Abdel Raouf [28]. The additional research should include different types of bio-oils derived from different biomasses.
- More research is required to validate the modifications of the Superpave test criterion and procedures recommended by Abdel Raouf [28].
- The resistance of the developed bio-binders against water and moisture intrusion should be studied. Moreover, the effect of water and moisture intrusion on the rheological properties should be investigated before the usage of the developed bio-binders as pavement materials.

- New means and methods to quantify the aging occurring in bio-binders should be studied extensively to establish a standard procedure or a specification to chemically quantify the aging taking place.

2.9 Environmental, Economic, and Energetic Perspectives

Most bituminous adhesives or binders that are used for pavement materials are derived mainly from fossil fuels. Nevertheless, with petroleum oil reserves becoming depleted and the subsequent impetus to reduce fossil fuel usage, there is a drive to develop and produce binders from alternative sources, especially from biorenewable resources. Importantly, the United States is working to establish a bio-based economy which generates energy from renewable organic matter rather than fossil fuels. Due to the availability of large quantities of biorenewable sources such as triglyceride oils, proteins, starch, and other carbohydrates from different botanical sources, there are virtuous technical and economic prospects in utilizing them to produce bio-binders. Recently, through the application of scientific research and development, a range of different vegetable oils have been investigated to determine their physical and chemical properties to study their applicability to be used as bio-binders in the pavement industry.

Currently, the state of the art for the utilization of bio-oils is concentrated on its uses as biorenewable fuels to replace fossil fuels. However, there is a limited amount of research that has been conducted to investigate the applicability of using bio-oils as a bitumen modifier or extender. Based on the conclusion of these investigations, the utilization of bio-oils as a bitumen modifier is very promising. On the other hand, there has been no research conducted until now that studies the applicability of the utilization of bio-oils as a bitumen replacement (100% replacement) to be used in the pavement industry. As a result, there is scarcity of data that illustrate the procedure to develop bio-binders from bio-oils. Bio-binders (synthetic binders) can be utilized in three different ways to decrease the demand for fossil-fuel-based bituminous binders summarized as follows: (1) as a bitumen modifier (< 10% bitumen replacement), (2) as a bitumen extender (25–75% bitumen replacement), and (3) as a direct alternative binder (100% replacement).

3 Bio-Polymers

The use of plastics in our everyday life is nearly endless. Due to its low cost of production and versatility, no alternate emerging product is likely to replace the nearly ubiquitous presence of plastics. The current global production level is about 250 million tons and its growth will continue to be robust globally. Plastics are preferred as they are light, durable, resist deterioration, and the markets they cater

to are extensive: food, textiles, furniture, electronics, vehicle parts, photography/videography, coatings, construction, enclosures, bottles/containers, and many more [29].

The most commonly used types of plastics are: polypropylene oxide (PO), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), phosphorylcholine (PC), polyurethane (PU), acrylonitrile-butadienestyrene (ABS), polyethylene (PE), polycarbonate (PC), polyacrylates, polyvinyl acetates, and polyamides. In the paving industry the most commonly used polymers are elastomers, e.g., styrene–butadiene–styrene (SBS) block copolymers. All the referred synthetic polymers are typically made from the naphtha fraction of petroleum or natural gas; and are heavy pollutants as they are not biodegradable [29].

None of these polymers are biodegradable and some of them are not even recyclable, causing environmental hazards. Additionally, the 'oil peak', i.e., the point at which more than half of the World's easily accessed oil will be consumed is approaching [30], with visible effects on crude prices.

Usually, plant-based biopolymers based on green sources cost more in energy and processing than conventional polymers. However, the increase on the price of petroleum has led to a dramatic increase in the price of the main constituents of polymers surpassing the price of some green sources of bio-polymers.

The high cost of biopolymers compared to traditional ones is not due to the raw material costs for biopolymer synthesis; rather it is mainly attributed to the low volume of production. [2]. Oils and fats (animal or vegetable in origin) have long been identified as possible substitutes for petrochemical derivatives in the production of polymers for many applications [1].

Over the next several decades, plant oils will become just as essential to everyday life as fossil fuels are today. This is why scientists are interested in making plastics from plant-based materials because they can produce an amazing array of compounds that could be used as the starting monomers to produce plastics. (The monomer is the individual molecule that, on polymerization, forms the polymer chain). Moreover, plant oils and fats have the same basic chemical structure as petroleum: both are made up of long chains of carbon atoms, e.g., hydrocarbons [1].

Biodegradable plastics and bio-based polymer products based on annually renewable agricultural and biomass feedstock can form the basis for a portfolio of sustainable, eco-efficient products that can compete and capture markets currently dominated by products based exclusively on petroleum feedstock [2].

3.1 Definition

Bio-plastics or organic plastics are a form of plastics derived from renewable biomass sources, such as vegetable oil, corn starch, pea starch, or microbiota,

rather than fossil-fuel plastics which are derived from petroleum. Some, but not all, bio-plastics are designed to biodegrade.

Biopolymers are polymers that are generated from renewable natural sources are often biodegradable, and not toxic to produce. According to the American Society for Testing and Materials (ASTM), biopolymers are degradable polymers in which degradation results from the action of naturally occurring micro-organisms such as bacteria, fungi, and algae [31].

Biopolymers are an alternative to petroleum-based polymers (traditional plastics) produced by living organisms. The field of biopolymers is still in its early stage, but is growing in popularity every day. The term biopolymer is often used for all polymers that are made from natural renewable resources and/or are completely biodegradable. Biopolymers can be produced by biological systems like micro-organisms, plants and animals, or chemically synthesized from biological starting materials (e.g. sugars, starch, natural fats or oils, etc.). Most of the biopolymers are biodegradable and some of them are even water soluble. Most of the biopolymers are compostable or will biodegrade in landfills, however the time can vary from a couple of days to even years, but they will eventually degrade [31].

The best example of a polymer that can be classified as a bio-polymer but due to its production process is not bio-degradable is natural rubber. Due to its unique characteristics, natural rubber is the most common elastomer in almost all human activities, raw or combined with synthetic elastomers. This elastomer has being applied in road pavements as a recycled material from tires, and it can improve pavements performance and durability.

The oldest commercial elastomer is natural rubber, which is made from the processed sap of a tropical tree. Natural rubber (NR) is a biopolymer which is known as polyisoprene. The rubber tree (Hevea brasiliensis) is the most common source of natural rubber used today. Polyisoprene can also be synthesized by polymerization from its monomer isoprene ($CH_2 = C(CH_3)CH = CH_2$), (IR). This is a rare example of a natural polymer that we can make almost as well as nature does [31].

3.2 Why Develop These New Materials?

The grade of the bitumen governs the performance of paving mixtures of in-service temperatures. In many cases, the characteristics of bitumen need to be altered to improve its elastic properties at low temperatures for sufficient cracking resistance and to increase its shearing resistance during sustained loads and high temperatures for rutting resistance. The physical properties of bitumen are typically modified with the addition of SBS polymers to produce an improved asphalt grade that enhances the performance of asphalt paving mixtures.

In 2008, there was a shortage of SB type polymers for the asphalt industry forcing asphalt mix producers and owner/agencies to search for different products

that can be used as a bitumen modifier. With the forecast of increasing demand of liquid asphalt into the next decade, the need of a cost-effective viable bitumen modifier that can be used in lieu of standard SB type modifiers will remain strong. Of the asphalt mixtures that are polymer modified, approximately 80% of polymer modified asphalt use SB type polymers. Thus a substantial market opportunity exists for creating new polymers that can supplement and/or replace SB type polymers used in asphalt pavements for bio-elastomers.

Chemistry and engineering teams are now working to transform renewable raw materials into new chemicals, polymers, formulated products, and composite materials, known as ''bio-materials''. When discussing bio-materials, it is important to note that plants do not cause any direct negative effects to the ecosystem, they can grow in different climatic zones, they recycle the carbon dioxide for the atmosphere of the Earth, and many work to improve soil fertility [1].

3.3 Primary Sources of Materials

Biopolymers may be obtained from renewable classified resources, synthesized microbially, or synthesized from petroleum-based chemicals. Through blend of two or more biopolymers, a new biopolymer may be designed for specific requirements [2].

Current bio-renewable plastics use crops such as corn or sugar beets and are usually manufactured through fermentation. A new sugar-based bioplastic can be sourced from non-food crops, these sugars are known as lignocellulosic biomass. Vegetable oils are renewable resources for industrial purposes and originate from five principal crops: soybean, palm oil, rapeseed, sunflower, and coconut [1].

Soybean represents over 30% of the 10 principal seeds. Besides acting as a food source, these oils also have wide industrial application, for example, soybean oil is attracting more attention for industrial application because it is readily available for large-scale production. More than 600 million pounds of soybean oil is produced annually in the United States, and are used for nonedible applications, including the production of industrial materials. More than half of this use of soybean oils falls in the category of fatty acids, soaps and feed. The remainder of the non-edible soybean market is used for the manufacture of inks, paints, varnishes, resins, plastics, and biodiesel [1].

Research on applications of soybean for non-food applications in plastics and composites is underway at various U.S. research institutions. Soybeans typically contain about 20% oil and 40% protein. Soy protein is available in three different forms, as soy flour, soy isolate, and soy concentrate. Both protein and oil from soybeans can be converted to plastic/resin. Chemically, soy protein is an amino acid polymer or polypeptide and soy oil is a triglyceride. Through extrusion cooking and blending technology, soy protein polymers are converted to biodegradable plastics and their biocomposites, whereas through functionalization of soy oil, resin suitable for natural fiber composites is produced [2].

3.4 Application of the Bio-Polymers

Biopolymers can be used in all kinds of applications, and with all kinds of production techniques. Some biopolymers can directly replace synthetic plastics in traditional applications, while others possess unique properties that may open new applications [31].

Biopolymers can be used for a lot of applications, from packaging to disposables, from diapers to cotton sticks, from car parts to bottles, etc. In theory conventional plastics may be substituted by biopolymers in many applications. In practice substitution is not always feasible, wanted, or the most economical way to use biopolymers. Besides technical development which is needed for some applications, the applications have to be economically feasible within a reasonable term. The economic feasibility depends on the investments needed for material and product development and the added value of the biopolymer in the application [31].

At present, important biosynthetic examples of biopolymers that have received much attention from an overall market perspective are the polylactides (PLAs), the general class of polyhydroxyalkanoates (PHAs), starch and starch blends, and cellulose derivatives. Supermarket chains in Europe are now becoming more interested in certain foodstuffs using biopackaging film based on PLA because of its transparency and barrier properties. Research on modifications of PLA to allow its use in a wider range of food packaging is ongoing [1].

In recent years, the use of polymers made from renewable materials has been developed in diverse areas especially for automotive or building components. In 2003, the Ford Motor Company showcased a biomaterials theme on the Model U concept vehicle. The Model U demonstrated ''green'' materials and manufacturing with a variety of technologies including polymer foams derived from soybeans for the seat cushions, a soy-based resin tailgate, corn-based tires, a corn-based compostable roof, and biolubricants [1].

The total sunk-in capacity for biopolymers in 2009 was around 500 million lbs. These included polylactide acid [PLA] (Nature Works, Galactic, Hycail BV); polyhydroxyalkanoates such as PHAs, PHB, and PHBH (Biomer, Procter & Gamble); polymers based on bio-based PDO (DuPont); cellulose polymers (Innovia Films); epoxy polymers from bio-glycerol; and starch polymers and blends (AkzoNobel [National Starch Chemical] and several other players). Nature Works (Cargill, Dow) is the major commercial player with a PLA capacity of 280 million lbs; and Novamont is the major producer of starch polymers and blends, with a capacity of 120 million lbs. The total capacity of biopolymers is expected to reach 1.3 billion lbs, if Braskem's 400 million lbs/year of bio-polyethylene production and Braskem's/Nova Zymes's 400 million lbs/year of bio-polypropylene production materializes in Brazil. At these levels, the biopolymer's share of the total global production of synthetic polymers will be a meager 0.26%. If biopolymers were to replace all of the polymer products, the amount of biopolymer

production would need to increase nearly 400-fold (from 1.3 billion lbs to over 520 billion lbs) [29].

3.5 Bio-Elastomers

Elastomers, or rubbery materials, have a loose cross-linked structure. Natural and synthetic rubbers are both common examples of elastomers. Elastomers possess memory, that is, they return to their original shape after a stress is applied. Elastomers are amorphous polymers and consist of long polymer chains above their glass transition temperature. The structure of elastomers is tightly twisted or curled [31]. An elastomer is a polymer with the property of viscoelasticity (colloquially "elasticity"), generally having notably low Young's modulus and high yield strain compared with other materials. The term, which is derived from elastic polymer, is often used interchangeably with the term rubber, although the latter is preferred when referring to vulcanizates.

Elastomers are very flexible and elastic, which means that they can undergo large elastic deformations without ruptures and recover substantially in shape and size after the load has been removed. Many elastomers can be stretched to several times their original length. Also, the cycle can be repeated numerous times with identical results, as with the stretching of a rubber band [31]. Elastomers are generally resistant to oil and fuel, impermeable to liquids and gases, but tend to deteriorate by oxidation [31]. Further, elastomers are either thermoplastic material (they can be re-melted) or thermoset material (that cannot be re-melted). Rubber is an older name for elastomers [31].

The use of plant-derived triglycerides represents a material for use in producing elastomers as they offer two reactive sites, the double bond in the unsaturated fatty acid chain, and the ester group. 90% of the derivatization reactions are carried out at the carboxylic group. Only 10% of oleochemical reactions involve the alkyl chain or the double bond. For most other uses, oils and fats must be split into the so-called oleochemical base materials: fatty acids, glycerol, and, as hydrogenation products of the fatty acid methyl esters, fatty alcohols. Oleochemicals are chemicals derived from biological oils or fats. Palm, soybean, rapeseed, and sunflower oil, as well as animal fats such as tallow, contain mainly long-chain fatty acids (e.g., C–18, saturated and unsaturated) and are used as raw materials for polymer applications and lubricants [1].

An example of a vegetable crop that contains a long-chain fatty acid, which makes the material more flexible, is epoxidized soybean oil (ESO). ESO possesses functional epoxy groups within the triglyceride molecule which can react with suitable reagents. Each triglyceride contains three fatty acid chains linked by a single glycerol molecule [1].

The fatty acid chains have 0–3 double bonds and vary in length from 16 to 22 carbon atoms. The ESO contains a long-chain fatty acid, which makes the material more flexible. They can be cross linked to form elastomeric networks and are

attractive raw material resource for polymer synthesis. Low-cost soy-based resins would offer a significant cost reduction through the partial replacement of epoxy resin for composite applications. Furthermore, vegetable oils are biodegradable and, therefore, environmentally friendly [1].

The conjugated soybean oil synthesized through the isomerization reaction of soybean oil transformed the structure of linoleic acid into conjugated linoleic acid structure, soy-based copolymer. These thermosets are thermally stable up to 250°C and that the bulk thermoset does not degrade until temperatures that slightly exceed 350°C. The range of properties attained with these materials makes them suitable for applications in which petroleum-based polymers are currently used [32].

The widespread use of non-biodegradable, petroleum-based polymeric materials has raised many environmental concerns. The demand for these non-renewable, virtually indestructible materials is increasing, as is the dependence on crude oil. A possible remedy is to use natural, renewable resources as feedstock for use in plastics [32].

Recently, a shift in interest toward making polymeric materials prepared from readily available, renewable resources, such as cellulose, starch, proteins, and natural oils, has been seen. These biopolymers offer the advantages of low cost, ready availability from renewable natural resources, and possible biodegradability. For years, however, modified vegetable oils have found applications in the plastics industry. Thus, the concept of using renewable resources in plastics is not new [32].

Much of the work on polymeric materials derived from natural oils has involved functionalized oils. Can et al. [33] and Cakmakli et al. [34] prepared a copolymer and a grafted copolymer from derivatives of soybean and linseed oils with styrene or methyl methacrylate by free-radical polymerization, respectively. Petrovic et al. [35] and [36] carried out the oxirane ring opening of epoxidized oils, followed by condensation polymerization with isocyanates to produce polyurethanes. Andjelkovic et al. [37] have primarily focused on developing thermosetting resins from non-functionalized natural oils, such as soybean oil, corn oil, linseed oil, tuna oil, fish oil, and a number of other natural oils via cationic, free-radical, and thermal polymerization [32].

Soybean oil (SBO) is biodegradable oil which is readily available in bulk from a renewable natural resource. It is a triglyceride oil composed of 4% stearic (C18:0), 25% oleic (C18:1), 52% linoleic (C18:2), 9% linolenic (C18:3) acids and 10% palmitic with approximately five carbon–carbon double bonds per triglyceride. The fatty acid chain containing varying number of double bonds, with many non-conjugated double bonds, which activity of free-radical polymerization is low. Andjelkovic et al. [37] have developed a variety of novel polymeric materials ranging from elastomers to tough, rigid plastics prepared by the cationic copolymerization of regular soybean oil, low-saturation soybean oil, and conjugated low-saturation soybean oil. These thermosets exhibit thermophysical and mechanical properties that are competitive with those of their petroleum-based counterparts. The advantages of these polymer materials are their low cost, availability from a renewable natural source and their possible biodegradability [32].

3.6 Bio-Elastomers and the Asphalt Technology

Poly(styrene–butadiene–styrene) (SBS): Poly(styrene–butadiene–styrene), is a hard rubber, and is therefore used in soles of shoes, tire treads, and other places where durability is important. It is a type of copolymer called a block copolymer. Its backbone chain is made up of three segments, where the first is a long chain of polystyrene, the middle a long chain of polybutadiene, and the last segment is another long section of polystyrene [31].

Polystyrene is a tough hard plastic, which gives SBS its durability, while polybutadiene is a rubbery material, and gives SBS its rubber-like properties. The material has the ability to retain its shape after being stretched and thus SBS is a unusual type of material called a thermoplastic elastomer [31].

Despite natural rubber being a constituent of tires, due to the vulcanization of the rubber, they cannot be recycled. Every year, approximately 9–10 kg of rubber tires per inhabitant are discarded in industrialized countries. Although tires are not regarded as a dangerous residue, their hollow shape usually brings sanitary problems and difficulties in their final disposal. When ignited, the resulting fire is impossible to be extinguished, releasing hazardous gases into the atmosphere. Finally, the vulcanized rubber of tires cannot be recycled or used for the same purpose as the unvulcanized polymer. Furthermore, society is not utilizing the valuable materials that exist in tires, namely its main constituent, the vulcanized natural and synthetic rubbers.

Thus, the introduction of crumb rubber in the production of asphalt-rubber (AR) mixtures for road pavements should be considered as a sustainable technology which will transform an unwanted residue into a new mixture with a high resistance to fatigue and fracture.

According to ASTM D 6814-02, rubber is a natural or synthetic elastomer that can be chemically cross linked/vulcanized to enhance its useful properties. Cross-linked rubbers or elastomers are three-dimensional molecular networks, with the long molecules held together by chemical bonds. They absorb solvent and swell, but do not dissolve. Furthermore, they cannot be reprocessed simply by heating [38].

There are two types of rubber: natural and synthetic. Natural rubber latex is obtained from the rubber tree called Hevea braziliensis. The raw rubber molecule is a long straight-chain isoprene hydrocarbon. The physical appearance of this hydrocarbon is of a spongy, flocculent nature. At temperatures below 100°C this spongy rubber becomes stiff and hard whereas when warmed above 100°C, it becomes flexible, soft, and transparent [39].

General purpose elastomers are hydrocarbon polymers. They include styrene-butadiene rubber (SBR), butadiene rubber (BR), and polyisoprene rubber—both natural rubber (NR) and synthetic rubber (IR). These “diene” rubbers contain substantial chemical unsaturation in their backbones, causing them to be rather susceptible to attack by oxygen, and especially by ozone. Additionally, they are readily swollen by hydrocarbon fluids. The primary application of these elastomers is in automobile and truck tires [38].

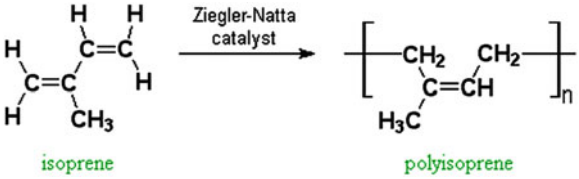

Fig. 3 Chemical structure, *cis*-1,4- polyisoprene [40]

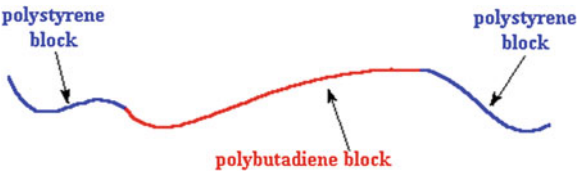

Fig. 4 Backbone chain of SBS

One of the most well known natural polymers is natural rubber. Ancient Mayans and Aztecs harvested it from the *Hevea* tree and used it to make waterproof boots and the balls which they used to play a game similar to basketball. It is an elastomer, that is, it recovers its shape after being stretched or deformed [40]. Today, NR is produced from the latex of the *Hevea brasiliensis* tree. Before coagulation, the latex is stabilized with preservatives (e.g., ammonia, formaldehyde, sodium sulphite) and hydroxylamine may be added to produce technically-specified, constant-viscosity grades of NR. The glass transition temperature, *Tg*, of NR is about –70°C and its structure is thought to be completely *cis*-1,4-polyisoprene (Fig. 3), except for the chain ends [38].

Polyisoprene is a diene polymer, which is a polymer made from a monomer containing two carbon–carbon double bonds. Like most diene polymers, it has a carbon–carbon double bond in its backbone chain. Polyisoprene can be harvested from the sap of the *Hevea* tree, but it can also be made by Ziegler–Natta polymerization. This is a rare example of a natural polymer that can be synthesized almost as well as nature does [40].

Poly(styrene–butadiene–styrene) or SBS (Fig. 4) is a hard rubber that is used for the soles of shoes, tyre treads, and other places where durability is important. It is a type of copolymer called a block copolymer. Its backbone chain is made up of three segments. The first is a long chain of polystyrene, the middle is a long chain of polybutadiene, and the last segment is another long section of polystyrene [40].

Polystyrene is a tough hard plastic, and this gives SBS its durability. Polybutadiene is rubbery, and this gives SBS its rubber-like properties. In addition, the polystyrene chains tend to clump together. When one styrene group of one SBS molecule joins one clump, and the other polystyrene chain of the same SBS molecule joins another clump, the different clumps become tied together with

rubbery polybutadiene chains. This gives the material the ability to retain its shape after being stretched [40].

SBS is also an unusual material called a thermoplastic elastomer. These are materials that behave like elastomeric rubbers at room temperature, but when heated, can be processed like plastics. Most types of rubber are difficult to process because they are cross linked. But SBS and other thermoplastic elastomers manage to be rubbery without being cross linked, making them easy to process into useful shapes [40].

The production of SBS rubber begins by using a technique called living anionic polymerization. A living polymerization is a polymerization that takes place without any termination reactions. This means that once all the monomer is used up, and has been turned into a polymer, yet the polymer chains are still active. Thus, if more monomers are added, the polymerization reaction continues and makes the polymers bigger [40].

Cross-linked materials can absorb solvents and thus a cross-linked material that has absorbed a lot of solvent is called a *gel* [40].

In practice, rubbers in contact with fluids will swell. The properties of the rubbers under swelling are also subject to dynamic or continuous changes [41]. The swelling process is fully reversible and there is no change in composition (such as removal of non-cross-linked or oligomeric components) of the rubber induced by the swelling process [42].

The effects of crumb rubber on asphalt-rubber binders can be separated into an interaction effect (IE) and a particle effect (PE). The IE is the effect of the lighter fractions of the binder diffusing into the crumb rubber particles. The PE is the effect of the crumb rubber particles acting as filler in the binder [43]:

- The IE is greatly influenced by the crude source of the binder and could potentially be used as an indicator of a binder's compatibility with CR (higher IE would indicate a more compatible binder);
- The PE is most significantly affected by the CR content of the binder. Higher CR contents result in greater PE values;
- The effect of CR size on the PE follows the same trends as either the viscosity or shear modulus (G*) of the CRM binders.

Gawel et al. [44] concluded that the rate of swell increases as the viscosity of the liquid decreases. The extent of swelling is greater when the rubber content in asphalt is lower. This finding suggests that it is not the whole asphalt material but only some of the components, those occurring in comparatively small amounts, which contribute to the swelling of the rubber and is primarily the lighter fractions that are involved. The lighter asphalt-vacuum residue blend components penetrate more readily into the internal matrix of the polymer. Asphalt-rubber binder (rubber removed) is richer in higher molecular weight components.

Gawel et al. [44] also found that, of the nonpolar components, the n-alkanes and n-alkylbenzenes possess the highest propensity to penetrate into rubber particles. Preferential absorption of the compounds with linear aliphatic chains into the

rubber suggests that these components have better skeletal compatibility with the linear polymeric skeleton of the rubber.

Green and Tolonen [45] emphasize the importance of controlling the swelling process through controlling the interaction time and temperature. They concluded that rubber particles absorb the lighter fractions of the "maltene phase" of the asphalt, so the viscosity of the "continuous phase" of the binder increases. Jensen and Abdelrahman [46] concluded that the swelling process may continue at a reduced rate even at ambient temperature.

Diffusion is assumed to follow Fick's law. Therefore, the rate of bitumen fractions transfer into rubber is proportional to their concentration gradient [47]. In the case of rubber and bitumen, as the rubber is, by definition, above its glass transition temperature, the polymer chains have considerable mobility so the system is essentially that of two fluid phases in contact but one phase has a network structure of polymer chains. So, in principle, when rubber and bitumen are placed in contact, low molecular weight components of the bitumen, i.e., aromatic oils, will diffuse into the rubber causing it to swell [48].

References

1. Rus AZM (2010) Polymers from renewable materials. Sci Prog 93(3):285–300
2. Mohanty AK, Misra M, Drzal LT (2002) Sustainable bio-composites from renewable resources: opportunities and challenges in the green materials world, J Polym Environ 10(1/2): 19–26
3. Demirbas MF, Balat M (2006) Recent advances on the production and utilization trends of bio-fuels: a global perspective. Energy Conserv Manag 47:2371–2381
4. Mohan D, Pittman CU, Steele PH (2006) Pyrolysis of wood/biomass for bio-oil: a critical review. Energy Fuels 20(3):848–889
5. Oasmaa A, Czernik S, Johnson DK, Black S (1999) Stability of wood fast pyrolysis oil. Biomass Bioenergy 7:187–192
6. Oasmaa A, Sipil K, Solantausta Y, Kuoppala E (2005) Quality improvement of pyrolysis liquid: effect of light volatiles on the stability of pyrolysis liquids. Energy Fuels 19(6): 2556–2561
7. Airey GD, Mohammed MH (2008) Rheological properties of polyacrylates used as synthetic road binders. Rheol Acta 47:751–763
8. Shields J (1976) Adhesives handbook. Butterworth, London
9. Tan CP, Che Man YB (2002) Comparative differential scanning calorimetric analysis of vegetable oils: effects of heating rate variation. Phytochem Anal 13:129–141
10. Kaplan DL (1998) Biopolymers from renewable resources. Springer, Berlin, pp 1–3
11. Dizhbite T, Telysheva G, Jurkjane V, Viesturs U (2004) Characterization of the radical scavenging activity of lignins-natural antioxidants. Bioresour Technol 95:309–317
12. Goyal HB, Seal D, Saxena RC (2006) Bio-fuels from thermochemical conversion of renewable resources: a review. Renew Sustain Energy Rev 12:504–517
13. Williams RC, Satrio J, Rover M, Brown RC, Teng, S (2009) Utilization of fractionated bio oil in Asphalt. In: DVD-ROM Proceedings of the 88th annual meeting of the transportation research board, Washington, DC
14. Ruan Y, Davison RR, Glover CJ (2003) Oxidation and viscosity hardening of polymer-modified asphalts. Energy Fuels 17:991–998

15. Domke CH, Dacidson RR, Glover CJ (2000) Effect of oxygen pressure on asphalt oxidation kinetics. Ind Eng Chem 39:592–598
16. Herrington PR, Patrick JE, Ball GFA (1994) Oxidation of roading asphalts. Ind Eng Chem Res 33(11):2801–2809. doi:10.1021/ie00035a033
17. Liu M, Ferry MA, Davidson RR, Glover CJ, Bullin JA (1998) Oxygen uptake as correlated to carbonyl growth in aged asphalt and corbett fractions. Ind Eng Chem 37:4669–4694
18. Ouyang C, Wang S, Zhang Y, Zhang Y (2006) Improving the aging resistance of styrene-butadiene-styrene tri-block copolymer modified asphalt by addition of antioxidants. Polym Degrad Stab 91:795–804
19. Kandhal PS (1992) Waste materials in hot mix asphalt—an overview, NCAT Report No. 92–6. Auburn University, Auburn, AL
20. Sundstrom DW, Klel HE, Daubenspeck TH (1983) Use of byproduct lignins as extenders in asphalt. Ind Eng Chem Prod Res Dev 22:496–500
21. Demirbas A (2008) Conversion of cornstover to chemicals and fuels. Energy Sources Part A: Recovery Util Environ Eff 30(9):788–796
22. Bothast RJ, Schlicher MA (2005) Biotechnological processes for conversion of corn into ethanol. Appl Microbiol Biotechnol 67:19–25
23. Van Dam EG, Klerk-Engles BD (2005) Resource supplies for changing market. Products 21:129–144
24. Diebold JP, Lilley A, Browne K, Walt RR, Duncan D, Walker M, Steele J, Fields M, Smith T (2008) Method and apparatus for automated, modular, biomass power generation. community power corporation, EP1896368, Patent. http://www.freepatentsonline.com/EP1896368A2.html. Accessed 26 June 2009
25. Bridgwater AV, Cottam ML (1992) Opportunities for biomass pyrolysis liquids production and upgrading. Energy Fuels 6(2):113–120
26. Mullen CA, Boateng AA (2008) Chemical composition of bio-oils produced by fast pyrolysis of two energy crops. Energy Fuels 22(3):2104–2109
27. Luo Z, Wang S, Liao Y, Zhou J, Gu Y, Cen K (2004) Research on biomass fast pyrolysis for liquid fuel. Biomass Bioenergy 26:455–462
28. Raouf MA (2010) Development of non-petroleum binders derived from fast pyrolysis bio-oils for use in flexible pavements. Dissertation, Iowa State University
29. Rao K (2010) The current state of biopolymers and their potential future. Omnexus by specialchem. http://www.omnexus.com/resources/editorials.aspx?id=25041. Accessed 30 March 2011
30. Rhodes CJ (2008) The oil question nature and prognosis. Sci Prog 91(4):317–375
31. Lukkassen D, Meidell A (2007) Advanced materials and structures and their fabrication processes. Book manuscript, Narvik University College, HiN, http://www.scribd.com/doc/24165910/26/Rubber-and-Arti%EF%AC%81cial-Elastomers. Accessed 10 April 2011
32. Yang L., Dai C., Ma L., Lin S (2010) Conjugation of soybean oil and it's free-radical copolymerization with acrylonitrile. J Polym Environ 1–7. doi: 10.1007/s10924-010-0259-z
33. Can E, Kusefoglu S, Wool RP (2001) Thermosetting liquid molding resins from renewable resources. I. Synthesis and polymerization of soy oil monoglyceride maleates. J Appl Polym Sci 81:69–77
34. Cakmakli B, Hazer B, Tekin IO, Kizgut S, Kosal M, Menceloglu Y (2004) Synthesis and characterization of polymeric linseed oilgrafted methyl methacrylate or styrene. Macromol Biosci 4:649–655
35. Petrovic ZS, Guo A, Zhang W (2000) Structure and properties of polyurethanes based on halogenated and nonhalogenated soypolyols. J Polym Sci A Polym Chem 38:4062–4069
36. Petrovic ZS, Zhang W, Zlatanic A, Lava CC, Ilavskyy M (2002) Effect of OH/NCO molar ratio on properties of soy-based polyurethane networks. J Polym Environ 10:5–12
37. Andjelkovic D, Valverde M, Henna PH, Li F, Larock RC (2005) Novel thermosets prepared by cationic copolymerization of various vegetable oils—synthesis and their structure–property relationships. Polymer 46:9674–9685

38. Hamed GR (1992) Materials and compounds. engineering with rubber: how to design rubber components from Alan N. Gent. Hanser Publishers, Germany
39. Rahman MM (2004) Characterisation of dry process crumb rubber modified asphalt mixtures. Dissertation, University of Nottingham
40. Polymer Science Learning Center (2005) *The macrogalleria—a cyberwonderland of polymer fun!* department of polymer science, the University of Southern Mississippi. http://www.pslc.ws/mactest/index.htm. Accessed 22 April 2008
41. Kumnuantip C, Sombatsompop N (2003) Dynamic mechanical properties and swelling behaviour of NR/reclaimed rubber blends. Mater Lett 57(21):3167–3174
42. Kariyo S, Stapf S (2004) NMR relaxation dispersion of vulcanized natural RUBBER. Solid State Nucl Magn Reson 25(1–3):64–71
43. Putman BJ, Amirkhanian SN (2006) Crumb rubber modification of binders: interaction and particle effects. In: Asphalt rubber 2006 conference, Palm Springs, USA, pp 655–677
44. Gawel I, Stepkowski R, Czechowski F (2006) Molecular interactions between rubber and asphalt. Ind Eng Chem Res 45(9):3044–3049
45. Green EL, Tolonen WJ (1977) The chemical and physical properties of asphalt-rubber mixtures. FHWA-AZ-HPR14-162, Arizona Department of Transport, Report ADOT-RS-14, 162
46. Jensen W, Abdelrahman M (2006) Crumb rubber in performance-graded asphalt binder. Nebraska Department of Roads, Final Report, SPR-01 (05) P585, University of Nebraska-Lincoln
47. Khalid HA (2005) Recent research on use of rubber in asphalt. WRAP rubber in roads seminar, University of Liverpool
48. Artamendi I, Khalid HA (2006) Diffusion Kinetics of Bitumen into Waste Tyre Rubber. In: Proceedings of the technical sessions, J Assoc Asphalt Paving Technol 75:133–164

Sustainable Utilization of Bio-fuel Co-Product in Roadbed Stabilization

Halil Ceylan, Sunghwan Kim and Kasthurirangan Gopalakrishnan

Abstract The sustainable utilization of bio-fuel co-products (BCPs) in construction application promises to support the viability of a growing bio economy and provides green construction practices. Biofuel production creates many different co-products depending on the method of biofuel production and co-products recovery technique and the biomass source. Among various BCPs, the industrial application of the BCP containing lignin has been limited although lignin is one of largest fractions of lignocellulosic biomass. This chapter discusses the utilization of BCP containing lignin in geotechnical engineering practices. Special attention is given to the experimental evaluations and pavement damage analysis to demonstrate the BCP containing lignin is a promising soil stabilization material, which can lead to dramatic breakthroughs for improving the durability, efficiency, economy, environmental impact, and sustainability aspects of civil engineering infrastructure systems.

1 Introduction

Sustainable development has been globally recognized under depleting non-renewable resources (petroleum, natural gas, coal, minerals, etc.), regulations for using synthetic materials, growing environmental awareness, and economic

H. Ceylan · S. Kim (✉) · K. Gopalakrishnan
Iowa State University, Ames, IA, USA
e-mail: sunghwan@iastate.edu

H. Ceylan
e-mail: hceylan@iastate.edu

K. Gopalakrishnan
e-mail: rangan@iastate.edu

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*, Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_7,

considerations [18]. Sustainable development requires safe, sustainable resources for various industrial applications [18]. Even though various alternative raw materials (wind, sun, water, biomass, nuclear fission, and fusion) may replace fossil-based energy, biomass is one of the most economical recourses, and its transformation into "bio-based products" and "bioenergy" through "biorefineries" in new production plants has the potential to replace petroleum-based refineries [18].

Bio-based energy production is often advocated as a significant contributor to possible solutions to our need for a sustainable transportation fuel. The burning of fossil fuels is a major contributor to atmospheric carbon dioxide [16]. As an alternative energy resource, the United States government has pushed significant industrial developments for using biomass [31, 33]. It is expected by 2020 that more than 90% of the consumption of organic chemicals in the United States and up to 50% of liquid fuel needs would be covered by bio-based products [18, 24]. The European Union (EU) has also requested member states to define national guidelines for a minimal amount of biofuels and other renewable fuels [18].

Biofuels are the most developed and widely used industrial application in the market from lignocellulosic biomass materials. Biofuel production from plant biomass also produces many different co-products that have many unexplored uses [2]. The type of co-products produced depends upon the method of biofuel production and co-products recovery method, as well as the source of biomass. The sustainable utilization of bio-fuel co-products (BCPs) in industrial application has been developed to support the viability of a growing bio economy.

This chapter is focused on issues that are related to use BCPs in civil engineering application to build a green civil infrastructure. Special attention is given to the roadway soil stabilization of geotechnical bulk application.

2 Bio-fuel Co-Product (BCP) and Roadway Soil Stabilization

2.1 BCP Containing Lignin

Biofuels are fuel products from biochemical and thermochemical conversion process of lignocellulosic biomass. The biofuels include bioethanol, bio oil, and biodiesel. Each biofuel product has its own biomass source and requiring processing for final fuel product [8]. Bioethanol is made from the fermentation of sugarcane and corn. Bio oil can be produced from various types of biomass from agricultural crops to agricultural and forestry by-products through thermochemical processes called as pyrolysis. Bio oil in itself has been utilized as liquid fuel for heat and power generator. It can be upgraded to transportation fuels although at present energetic and financial efficiency is still improving [6]. Bio diesel is made through the transesterification process which mixes organically derived oils and ethanol with catalyst. The derived oils for bio diesel production are extracted from plant oils and animal fats.

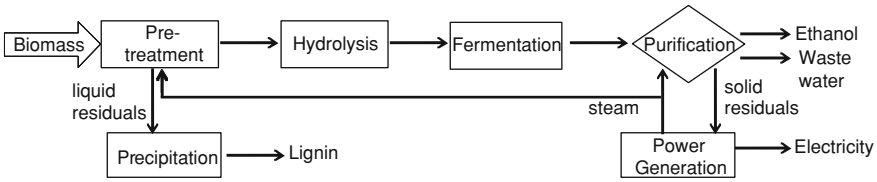

Fig. 1 Generalized biomass-to-ethanol process diagram [15]

Lignocellulosic biomass as feedstock of biofuel production mainly consists of cellulose, hemicellulose, and lignin. Lignocellulosic biomass can be converted into bioethanol by hydrolysis and subsequent fermentation [15]. In hydrolysis, the cellulosic part of the biomass is converted into fermentable sugars. To increase the yield of hydrolysis, a pretreatment step that softens the biomass and breaks down cell structures to a large extent is required. The pretreatments not only make the cellulose component susceptible to saccharification, but also have the potential of generating sulfur-free lignin with the hemicellulose as a residue [15]. Figure 1 presents the generalized biomass-to-ethanol process.

The pretreatment methods can be classified into physical, chemical, or biological. The physical pretreatment is to clean and size the biomass and destroy its cell structure to make it more accessible to further chemical or biological treatment. Desired sizes of the biomass after physical treatment vary from a few centimeters [34] to 1–3 mm [22]. Common chemical pretreatment methods use dilute acid, alkaline, ammonia, organic solvent, sulfur dioxide, carbon dioxide, or other chemicals. Biological pretreatments use fungi to solubilize/degrade the lignin [13]. Biodelignification is the biological degradation of lignin by microorganisms. Biological pretreatment has the advantage of low energy use and mild environmental conditions. However, the very low hydrolysis rate impedes its implementation [29]. Biological treatments can sometimes be used in combination with chemical pretreatments [13].

Bio oil refers to a dark brown, free-flowing organic fluid producing from fast pyrolysis in which biomass is rapidly heated in the absence of oxygen [23]. Figure 2 presents the generalized biomass-to-bio oil process. The raw bio mass materials are dried to a water content of typically less than 10% and grounded ground to 1 to 2 mm particle size. The prepared biomass is fed into the pyrolysis reactor with high heating rates, at controlled temperature of around 500°C and with short vapor residence times (typically less than 2 s) [3]. In pyrolysis reactor, biomass decomposes into gases and solid materials (char). The resulting gases pass into a cyclone where solid particles are extracted. The collected gases can be transformed into liquid type of bio oil after cooling and condensation of the gases. Even though the bio oil compositions are different as depending on source lignocellulosic biomass, the bio oil generally contains about 25% of lignin and up to 25% of water. The water component in bio oil for use of liquid fuel is not a separate phase because it lowers the viscosity of the fuel [9]. The water soluble

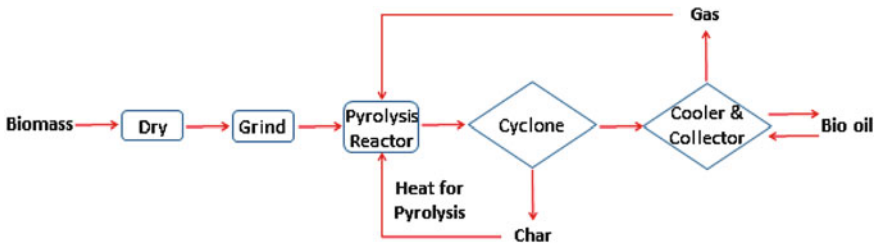

Fig. 2 Generalized biomass-to-bio oil process diagram

parts of bio oil are the most carbohydrate derived compounds while the water insoluble parts of bio oil are the pyrolytic lignins which are sulfur-free lignins [6].

Biofuel production creates many different co-products that have many unexplored uses [2]. The type of co-products produced depends on the method of biofuel production and co-products recovery technique and the biomass source. Among many different co-products, lignin, which represents the third largest fraction of lignocellulosic biomass, has been considered as a waste material or a low-value co-product, with its utilization predominantly limited to use as a fuel in the production of octane boosters and in bio-based products and chemical production [28]. It is therefore extremely important to recover and convert biomass-derived lignin into high-value product for the profitability of the bio-based products and the bioenergy business [10].

2.2 Lignin as a Roadway Soil Stabilizer

A good road (paved or unpaved) requires a suitable foundation, which in turn requires soil stability. The degree of stability is primarily a function of the road material resistance to lateral movement or flow [32]. Different types of road material employ different mechanisms for resisting lateral movement. In general, granular soils count on their particle sizes, angularity, and interlocking ability to develop the internal friction required to resist lateral flow. However, in fine-grained soils such as clay soils, the stability is very much moisture-dependent. As a result, well-established techniques of soil stabilization are often used to improve the properties of geotechnical materials through the addition of binding agents into soil [1].

Soil stabilization in geotechnical engineering refers to the process of blending and mixing materials with a soil to increase the shear strength of that soil corresponding to the given requirements [17, 19]. The soil-stabilizing additives or admixtures traditionally used include hydrated lime, portland cement, and fly ash.

The use of lignin in soil stabilization has been studied over the past decades [10]. Adding lignin to clayey soils increases the soil stability by causing dispersion

of the clay fraction [7, 12]. According to [12], the dispersion of the clay fraction benefits stability of the soil-aggregate mix by: a) plugging voids and consequently improving water tightness and reducing frost susceptibility, b) eliminating soft spots caused by local concentrations of binder soil, c) filling voids with fines thus increasing density, and d) increasing the effective surface area of the binder fraction which results in greater contribution to strength. Lignin is also used in combination with other chemicals to achieve soil improvement [27].

In most of the previous studies described, natural lignin or sulfite lignin (lignosulfonates) has been utilized. However, the lignin in biofuel production is sulfur-free. Even though sulfur-free lignins have been known for many years, the use of sulfur-free lignin has recently gained interest as a result of diversification of biomass processing schemes [21]. It has been hypothesized that since the BCP derived from plant biomass is high in lignin (sulfur-free lignin), which is thought to play a role in stabilizing soil, soil incorporation of BCP may help maintain or improve soil structure and stability [10].

3 Application of BCP in Pavement Geo-Materials Stabilization

Recently, the researchers at Iowa State University (ISU) began investigating the effectiveness of BCP as a stabilization material for roadway geofoundations [4, 5, 11]. Laboratory tests were conducted to evaluate the strength performance, soil engineering properties, and the moisture susceptibility of two types of BCP-treated soil samples compared to those of untreated and traditional stabilizer-treated (fly ash) soil samples. In conjunction with laboratory studies, the ISU researchers investigated the impact of BCP stabilized subgrade on Hot Mix Asphalt (HMA) pavement performances using the mechanistic-based damage analysis system. This section summarizes the ISU research approaches and results focusing on the sustainable use of BCP in geotechnical applications.

3.1 Laboratory Experimental Evaluation

3.1.1 Materials

Natural soils were collected from a new construction site for US 20 in Calhoun County, Iowa. The collected soil samples can be classified as an A-6(8) soil and CL in accordance to the American Association of State Highway and Transportation Officials (AASHTO) soil classification system and Unified Soil Classification System (USCS), respectively.

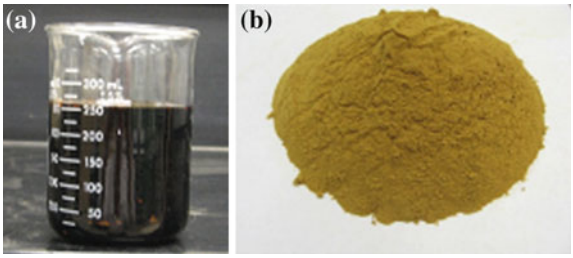

Fig. 3 Biofuel Co-Product A (*left*) and Co-Product B (*right*)

Two types of BCPs containing lignin were used as additives and designated as co-products A and B. Co-product A, shown in Fig. 3(left), was obtained from a commercial biomass conversion facility located in Canada [9]. This BCP is a bio oil containing about 25% lignin and up to 25% water with a pH value of 2.2. It is formed in a process of fast pyrolysis. The water component in co-product A to be used as liquid fuel is not a separate phase because it lowers the viscosity of the fuel. Co-product B, shown in Fig. 3(right), was obtained from a full-scale, wet-mill, corn-based ethanol plant located in Iowa [14]. Alkaline-washed corn hull is obtained in the process of converting the corn into ethanol, and co-product B is a powdered version of this. Co-product B contains about 5% lignin, 50% hemicellulose, 20% cellulose, and other components. These lignin-type components are not high molecular weight lignin like those found in wood but are specific to maize. The Ottumwa class C fly ash was selected as the traditional additive to compare biofuel co-products' relative performance.

3.1.2 Effect of BCPs on Strength Performance

The stabilization effect of the soil additive is measured in terms of the increase in load-bearing capacity as indicated by unconfined compression test (UCS) [20, 26, 30]. The effects of co-product types and contents on UCS were evaluated under different moisture conditions: OMC represented moisture condition providing the maximum dry density of soil and used for quality control of construction, OMC − 4% represented the more dry side of soil condition, and OMC + 4% represented the more wet side of soil condition. The evaluations were also made under different curing periods. The results are shown graphically in Fig. 4.

The UCS values at 0% additive content in this figure indicate untreated soil after one and seven days of curing. The strengths of untreated soils are in all cases lower than the strengths of additive-treated soils. Overall, each of the BCPs-treated soil UCS test results shows strength improvements. However, the strength improvements of the co-products-treated soil are not higher than those improvements of the fly ash-treated soil. The strengths of treated soil increase with the increase in co-product concentrations and curing periods. A high increase in strengths for the BCPs-treated soil occurs with 12% of co-product A in all cases.

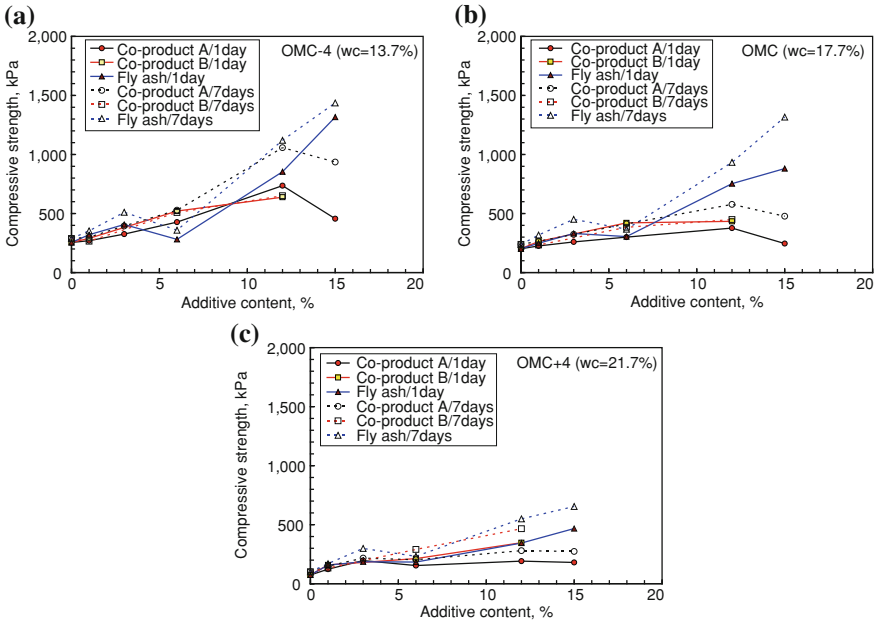

Fig. 4 Effect of traditional and BCP soil stabilizers on soil strength: **a** wetter side; **b** optimum; **c** drier side

The strengths under the more dry side of soil condition are higher than the more wet side of soil condition. All the results under different moisture conditions indicate that co-product A is more effective in improving strength under dry conditions while co-product B is more effective in improving strength under wet conditions.

3.1.3 Effect of BCPs on Soil Engineering Properties

Soil samples prepared with additives at selected percentages were subjected to engineering properties tests to determine their physical properties and compaction characteristics. Engineering properties tests included the consistency limits (liquid limit [LL] and plastic limit [PL]) and the moisture/density relationship. The final selected percentage of co-products was one at which the compression strength values were maximum. 12% of co-product content was selected in this evaluation because a high increase in UCS occurred with 12% of co-products.

Figure 5 presents the effect of additives on consistency limits and compaction properties of soil. The co-product A reduced the plasticity of soils as a result of an increase in the plastic limit value, but the co-product B increased the plasticity of soils as a result of an increase in the liquid limit and plastic limit values. Co-product

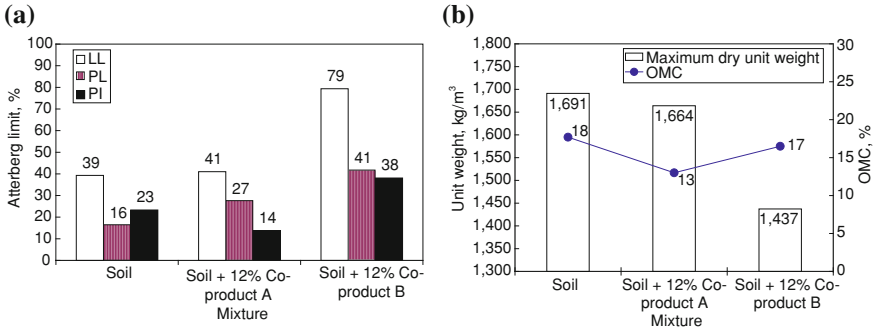

Fig. 5 Effect of BCP soil stabilizers on soil engineering properties: **a** consistency limits; **b** compaction properties

A decreases the OMC with 1,664 kg/m^3 of the maximum dry unit weight when compared to the maximum dry unit weight of untreated soil. However, co-product B decreases the maximum dry unit weight with 17% of OMC when compared with the maximum dry unit weight of untreated soil. These results indicate that co-product A is a more promising additive, considering the reduction in the plastic property and the decrease in OMC with increasing maximum dry unit weight as indicators of improvement for soil stabilization purposes.

3.1.4 Effect of BCPs on Moisture Susceptibility

The effect of lignin-based BCPs on moisture susceptibility of subgrade soil after compaction was investigated. The laboratory experimental program was conducted using two types of tests, which were the UCS tests after "dry" and "wet" pre-conditioning procedure and the visual observations of soaked specimens (so-called soaking tests). The UCS test was applied to dry and wet specimens evaluate moisture susceptibility of additive-treated specimens. Specimens were also fully soaked into water over a period of time to examine if specimens would fail due to moisture damage and at what time periods.

The results of UCS tests under dry and wet pre-conditions are shown graphically in Fig. 6. The "0" value in Fig. 6 indicates that a specimen disintegrated when exposed to water. The untreated soil specimen was used as control specimen. Overall, the strengths of additives treated soils are in all cases higher than untreated soils under the dry and wet conditions. The fly ash treated soil test results show the most improvement of UCS under the dry conditions. However, the fly ash treated soil specimens provided more strength reduction after wet pre-condition compared to the BCPs treated soil specimens.

Apart from the UCS tests, the compacted specimens of each mixture were subjected to so-called soaking tests. The objective of these tests was to evaluate the

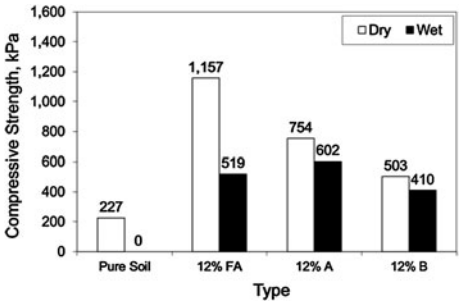

Fig. 6 UCS test results for dry and wet specimens

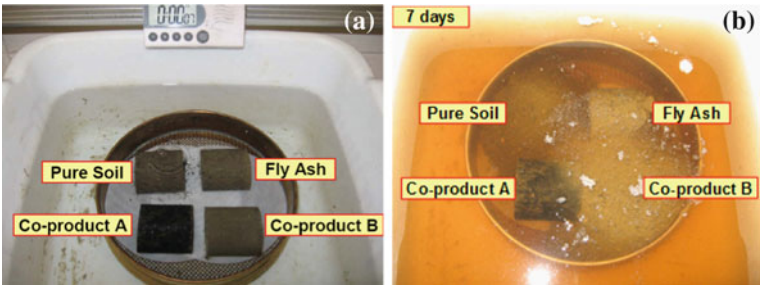

Fig. 7 Soaking test results: **a** initial state; **b** after 1 day; **c** after 7 days

long-term moisture susceptibility of specimens treated without and with additives, and to determine when specimens disintegrated due to water. Specimens were fully soaked into water as seen in Fig. 7. Two test sets of specimens were prepared for these tests. Test set 1 included untreated soil (pure soil), 12% of fly ash treated soil, 12% of co-product A treated soil, and 12% of co-product B treated soil specimen. The failures of specimens were observed during 7 days after soaking.

As seen in Fig. 7 (b), the fly ash-treated soil specimen and co-product B-treated soil specimen disintegrated at day seven after soaking. However, the deterioration of the specimens after seven days of soaking did not occur in soil specimens treated with co-product A. These soaking test results demonstrate that the BCP A–treated soil specimens do not deteriorate, even after long-term moisture exposure. The BCP A can provide excellent waterproofing for clay soils.

3.2 Mechanistic-Based Pavement Damage Analysis

Mechanistic-Empirical Pavement Design Guide (MEPDG) was utilized as the mechanistic-based damage analysis system to predict distress measurements of HMA pavement with BCP stabilized subgrade against natural subgrade performance during pavement service life. The MEPDG and its software were developed

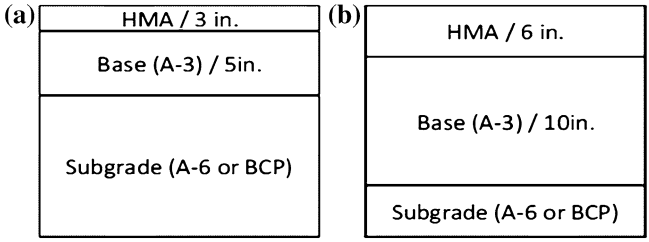

Fig. 8 Pavement structure modeled; **a** low traffic, **b** high traffic

to provide more rational methodologies in pavement thickness design through National Cooperative Highway Research Program (NCHRP) 1-37A project [25]. The MEPDG employs the application of the principles of engineering mechanics to calculate pavement responses (stresses, strains, and deflection) under loads for the predicting the pavement performance history.

The HMA pavement systems with BCP A stabilized subgrade was modeled under different traffic and climatic conditions. The HMA pavement systems with natural soil subgrade were also modeled for each case. Four sites representing different climate extremes in US were considered: Des Moines in Iowa (wet - freezing and thaw zone), Orlando in Florida (wet - no freeze zone), Billings in Montana (dry-freezing and thaw zone), and San Antonio in Texas (dry-No freeze). Typical asphalt performance grade utilized in each site was assigned corresponding HMA material property inputs: PG 58-28 for Des Moines in Iowa, PG 67-22 for Orlando in Florida, PG 64-28 for Billings in Montana, and PG 70-16 for San Antonio in Texas. Two levels of initial Average Annual Daily Truck Traffic (AADTT) considered were 100 and 12,000 vehicles per day representing low and high traffic levels. A 5% of compound traffic growth rate was set up over a 20-year design life. Two typical HMA pavement structures, shown in Fig. 8, were modeled for low and high traffic level cases.

The performance measures investigated were alligator cracking and rutting. Figure 9 illustrates the predicted alligator cracking and rutting with service times of the modeled high traffic volume HMA pavements in Iowa (wet-freezing and thaw zone) to disuse the impact of BCP A stabilized subgrade on HMA pavement performance measures. As seen in this figure, the HMA pavement with BCP-treated subgrade had much lower alligator cracking and rutting predictions than one with natural subgrade. This behavior could be explained by the higher resilient modulus of BCP stabilized soil materials, i.e., soil strength and stiffness improvement caused by BCP could contribute to prevent pavement distress during service life.

Figure 10 and 11 summarize the predicted alligator cracking and subgrade rutting for low and high traffic volume HMA pavements at each of the four climate locations. It is clear, according to these figures, that all of traffic levels and climate locations investigated have lower alligator cracking and subgrade rutting of BCP-treated pavement rather than untreated pavement. The MEPDG performance

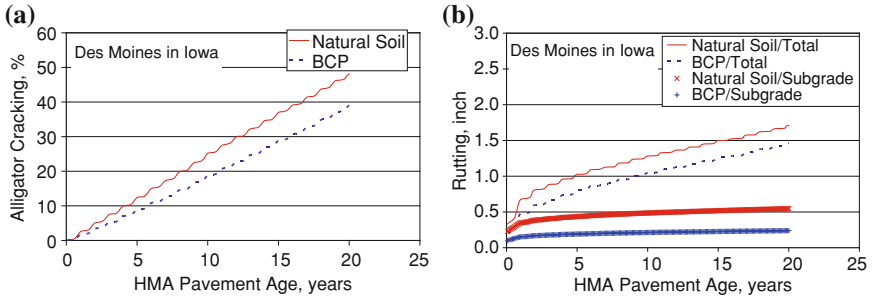

Fig. 9 Distress predictions with age of modeled high traffic volume HMA pavements in Iowa; **a** alligator cracking, **b** rutting

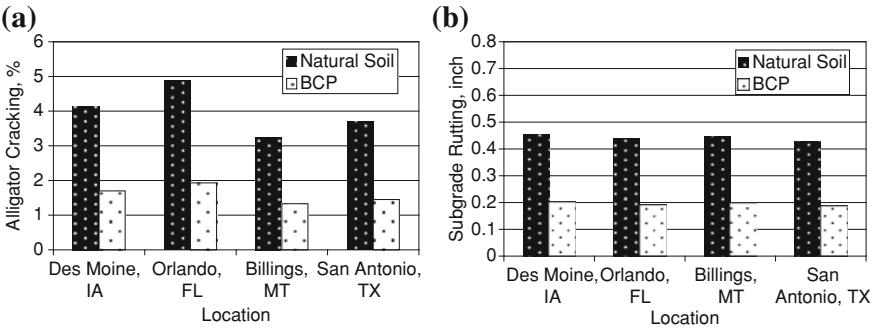

Fig. 10 Low traffic volume HMA pavement performance predictions after 20 years' service life: **a** alligator cracking, **b** subgrade rutting

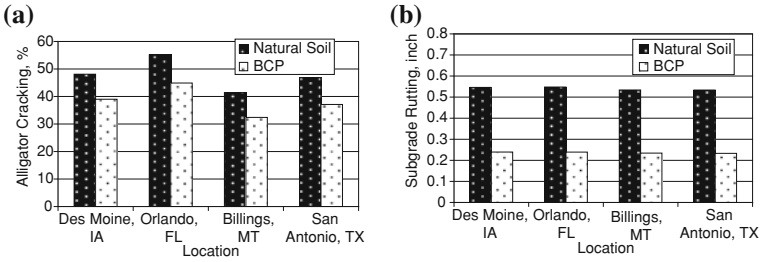

Fig. 11 High traffic volume HMA pavement performance predictions after 20 years' service life: **a** alligator cracking, **b** subgrade rutting

predictions of these figures suggest that BCP stabilization of subgrade soil could achieve more sustainable HMA pavements under different traffic volume and climate conditions.

4 Summary

The increasing cost of fossil-based energy with the problems of global warming has being driving increased bio-based energy production from plant biomass as renewable energy. Sustainable utilization of BCPs in industrial and construction applications is imperative to support the viability of a growing bioeconomy and a green civil infrastructure. Experimental laboratory test results demonstrate that BCPs are promising materials to improve roadbed strength and engineering properties, and provide excellent resistance to moisture degradation of low quality of soil materials. The pavement damage analysis indicates that BCP stabilization of subgrade soil could help achieve long-lasting pavements. Considering the wide range of applications in which BCPs could be used, more research is needed to enable broader use of BCPs as alternative construction materials which can lead to dramatic breakthroughs for improving the durability, efficiency, economy, environmental impact, and sustainability aspects of civil engineering infrastructure systems.

References

1. Basha EA, Hashim R, Mahmud HB, Muntohar AS (2005) Stabilization of residual soil with rice husk ash and cement. Constr Build Mater 19:448–453
2. Bothast RJ, Schlicher MA (2005) Biotechnological processes for conversion of corn into ethanol. Appl Microbiol Biotechnology 67(1):19–25
3. Bridgwater AV, Meier D, Radlein D (1999) An overview of fast pyrolysis of biomass. Org Geochem 30:1479–1493
4. Ceylan H, Gopalakrishnan K, Kim S (2009) Use of bio-oil for pavement subgrade soil stabilization. In Proceedings of Mid-Continent Transportation Research Symposium. CD-ROM. Iowa State University, Ames, IA
5. Ceylan H, Gopalakrishnan K, Kim S (2010) Soil stabilization with bioenergy co-product. Trans Res Rec: J Trans Res Board 2186:130–137
6. Czernik S, Bridgwater AV (2004) Overview of applications of biomass fast pyrolysis oil. Energy and Fuels 18:590–598
7. Davidson DT, Handy RL (1960) Section 21: soil stabilization. In: Wood KB (ed) Highway Engineering Handbook. McGraw-Hill, NY
8. Demirbas A (2007) Progress and recent trends in biofuels. Prog Energy Combust Sci 33:1–18
9. Dynamotive Energy Systems Corporation (2007) Dynamotive BioOil Information Book. Vancouver
10. Gopalakrishnan K, Kim S, Ceylan H (2010a) Lignin utilization and recovery. In: Khanal S (ed) Biofuel and Bioenergy from Biowastes and Lignocellulosic Biomass. American Society of Civil Engineers (ASCE) Press. Reston, VA
11. Gopalakrishnan K, Ceylan H, Kim S (2010b) Biofuel co-product uses for pavement geomaterials stabilization, IHRB Project Final Report. The Institute for Transportation, Ames, IA
12. Gow AJ, Davidson DT, Sheeler JB (1961) Relative effects of chlorides. lignosulfonates and molasses on properties of a soil-aggregate mix, Highway Research Board Bulletin, p 282
13. Graf A, Koehler T (2000) Oregon Cellulose-Ethanol Study. Oregon Office of Energy, Salem
14. Grain Processing Corporation (GPC). (2009) Muscatine, IA., Available via http://www.grainprocessing.com/. Cited 5 March 2009

15. Hamelinck CN, Hooijdonk GV, Faaij APC (2005) Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle-and long-term. Biomass Bioenergy 28(4):384–410
16. IPCC (1996) Climate change 1995: the science of climate change: the contribution of working group I to the second assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge
17. Joint Departments of the Army and Air Force (JDAAF) (1994) Soil Stabilization for Pavements. TM 5-822-14/AFMAN 32-8010, USA
18. Kamm B, Kamm M (2004) Principles of biorefineries. Appl Microbiol Biotechnology 64(2):137–145
19. Kezdi A (1979) Stabilized earth roads. Elsevier/North-Holland, Inc., New York
20. Lane DD, Baxter TE, Cuscino T, CJr Coward (1984) Use of laboratory methods to quantify dust suppressants effectiveness. Trans Soc Min Eng American Inst Min, Metall, Petroleum Eng 274:2001–2004
21. Lora JH, Glasser WG (2002) Recent industrial applications of lignin: a sustainable alternative to nonrenewable materials. J Polym Environ 10(1–2):39–48
22. Lynd LR (1996) Overview and evaluation of fuel ethanol from cellulosic biomass technology, economics, the environment, and policy. Annu Rev Energy Environ 21:403–465
23. Mohan D, CUJr Pittman, Steele PH (2006) Pyrolysis of wood/biomass for Bio-oil: a critical review. Energy Fuels 20:848–889
24. National Research Council (2000) Biobased industrial products, priorities for research and commercialization. National Academic Press. Washington, DC
25. RP NCH (2004) Guide for mechanistic-empirical design of new and rehabilitated pavement structures. Draft Final Report NCHRP Project 1–37A. Transportation Research Board. National Research Council, Washington, DC
26. Palmer JT, Edgar TV, Boresi AP (1995) Strength and Density Modification of Unpaved Road Soils due to Chemical Additives. Mountain-Plains Consortium (MPC) Report No. 95–39. Fargo
27. Puppala AJ, Hanchanloet S (1999) Evaluation of a chemical treatment method (sulphuric acid and lignin mixture) on strength and resilient properties of cohesive soils. Paper presented at 78th Transportation Research Board Annual Meeting. Washington, DC CD ROM
28. Stewart D (2008) Lignin as a base material for materials applications: chemistry, application and economics. Ind Crops Prod 27(2):202–207
29. Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production a review. Bioresource Technol 83(1):1–11
30. Tingle JS, Santoni RL (2003) Stabilization of clay soils with nontraditional additives. Transp Res Rec: J Transp Res Board 1819:72–84
31. US Congress (2000) Biomass research, development act of 2000. U.S. Congress, Washington, DC
32. US DOT (1976) America's Highways:1776–1976. Federal Highway Administration. U.S. Department of Transportation, Washington, DC
33. U.S. President (1999) Developing and promoting bio-based products and bioenergy (Executive order 13101/13134, William J. Clinton). The White House, Washington, DC
34. Wooley R, Ruth M, Sheehan J, Ibsen K, Majdeski H, Galvez A (1999) Lignocellulosic biomass to ethanol—process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hyrolysis—current and futuristic scenarios. Report No. TP-580-26157, National Renewable Energy Laboratory, Golden

Microbial Fuel Cells for Bioenergy and Bioproducts

Minghua Zhou, Tao Jin, Zucheng Wu, Meiling Chi and Tingyue Gu

Abstract Redox reactions are essential in microbial bioenergetics. Oxidation of an organic carbon and reduction of an oxidant such as oxygen, sulfate and nitrate in the cytoplasm of a microbe. This respiration process provides energy for various cellular activities and maintenance as well as building blocks for organic syntheses. Because the cellular respiration efficiency is around 40, 60% of the energy is released in the form of unrecoverable low-grade heat. No electricity is produced from the redox reaction because the electrons released by the oxidation reaction are taken up locally by the reduction reaction in the cytoplasm. The purpose of a microbial fuel cell (MFC) is to harvest the electrons from organic carbon oxidation by employing electrogenic microbes to donate the electrons to an anode in the absence of an utilizable oxidant around the anode. The electrons flow through an external circuit to drive a load before returning to a cathode where they are used for reduction of an oxidant. MFCs can digest low-grade organic carbon sources while yielding high Coulombic efficiencies. They present a potentially attractive alternative for the sustainable production of bioenergy and bioproducts from renewable organic feed streams through biocatalysis by microorganisms. In recent years, heightened concerns over depleting fossil fuel supplies, most noticeably petroleum, and global

M. Zhou · T. Jin · M. Chi
Key Laboratory of Pollution Processes and Environmental Criteria of Ministry of Education, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

Z. Wu
Department of Environmental Engineering, Zhejiang University, Hangzhou 310027, China

T. Gu (✉)
Department of Chemical and Biomolecular Engineering, Ohio University, Athens, OH 45701 USA
e-mail: gu@ohio.edu

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*, Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_8,

warming due to increased carbon emission, many efforts have been devoted to MFC research to debottleneck various factors that hamper practical applications of MFCs. Although no large-scale applications have been reported, the improvements in MFC designs and operations have made MFCs closer to eventual practical applications in the production of bioenergy and bioproducts from feed streams that are typically wastes such as wastewater. This chapter reviews various advances in the understanding of MFC mechanisms, reactor designs and operations for improved production of renewable bioenergy and bioproducts such as hydrogen, methane, hydrogen peroxide and ethanol. The limitations, areas for improvement and perspectives for future outlooks of MFCs are also discussed.

1 Introduction

1.1 Energy Crisis and Environmental Pollution

Abundant and cheap energy is viewed as the lifeblood of any modern economy. The current global energy consumption relies mostly on fossil fuels, that are primarily oil, natural gas and coal [116]. Political and industrial leaders have realized that these non-renewable energy resources are unsustainable [109, 173], particularly in view of the rapid growth in energy consumption by emerging economic powers such as China and India. Affordable energy is a major challenge facing human societies [75], and it will be a bottleneck for economic development and will likely dominate our modern lifestyle [109].

On the other hand, the release of carbon, sulfur, nitrogen from combustion of fossil fuels increases the concentrations of carbon oxides, sulfur oxides and nitric oxides in the atmosphere, causing environmental pollution that threatens human societies. Apart from concerns over global warming and atmospheric pollutions due to fossil fuels, water pollution is another serious issue [41]. Clean water is essential to human activities [133]. The energy consumption in municipal wastewater treatment is significant, accounting for approximately 1.5% of municipal electricity in the US [109].

In the twenty-first century, a sustainable energy supply is necessary for economic progress [121, 194]. This provides an impetus for a gradual shift from fossil fuels to renewable energy resources such as solar, wind, biomass, and geothermal. The ultimate success of the transition depends on whether these green technologies are technically and economically competitive.

1.2 Microbial Fuel Cells (MFCs)

Microbial fuel cells (MFCs) can be defined as systems that use microbes as the biocatalysts to oxidize substrates (mostly organic and occasionally inorganic) and

reduce an oxidant to generate a current via biochemical pathways [12, 104, 138]. The microbial respiration chain consisting of organic carbon oxidation and oxidant reduction are split into anodic and cathodic reactions in order to generate electricity. Both aerobic respiration and anaerobic respiration can be utilized. In 1911, Potter first managed to generate electricity from an *Escherichia coli* culture [155]. However, this discovery did not generate much attention because the voltage and current from his cell were miniscule. Cohen [32] subsequently proved repeated the results and developed stacked microbial fuel cells capable of generating voltages great than 35 V. Microbial electricity generation was revived in the 1960s due to a geopolitically motivated push by the NASA space program [187]. The recent energy crunch and growing awareness of global warming have reinvigorated MFCs as a potentially useful green energy technology beyond their current practical applications as biosensors and as a power supply for remote sensors.

MFCs have the distinct advantage of using low-grade biomass such as wastewater for energy generation [139]. Various substrates, from simple volatile fatty acids such as acetate to complex biopolymers such as lignocellulose, can be utilized by MFCs as fuels. Domestic, industrial and agricultural waste streams can all potentially be low-cost or zero-cost feed stocks for MFCs [139, 176].

As an example, the following two electrode reactions explain the working principle of an MFC with acetate as the organic substrate (reducer) and oxygen as the oxidant.

$$\text{Anode}: \ CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^- \tag{1}$$

$$\text{Cathode}: \ O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \tag{2}$$

If acetate is oxidized and oxygen is reduced in the microbial cytoplasm, no electrons care released. An MFC is used to split the redox reaction. In the anodic chamber, an anaerobic biofilm oxidizes acetate and donates the electrons to the anode. Through an external circuit, the electrons flow from the anode to the cathode where the electrons are used to reduce oxygen. Electro-neutrality of the system is maintained because protons migrate from the anode to the cathode in the aqueous solution through a proton exchange membrane (PEM). The overall reaction is the acetate conversion to CO_2 and water [38]. It is thermodynamically favorable (i.e., exothermic), but kinetically retarded. Biocatalysis by the anode biofilm moves the anodic reaction forward, while platinum cathode and/or an electron mediator in the catholyte solution catalyzes the cathodic reaction.

Figure 1 shows a dual-chamber MFC with an anaerobic biofilm covering its anode. Electrons produced by the microorganisms from organic carbon oxidation flow from the anode to the cathode to drive an external load. They are subsequently used to reduce oxygen in the cathodic chamber [104, 164].

Because MFCs can utilize many different kinds of organic matters that are typically low-grade, they are considered a potential technology for waste treatment with concomitant bioenergy production [104, 217]. MFCs can also be used to

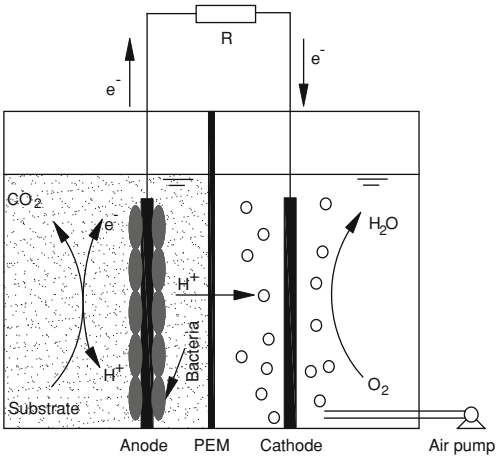

Fig. 1 Schematic of a microbial fuel cell

produce different bioproducts such as hydrogen and methane. This book chapter is a critical review of various aspects of the MFC technology.

2 MFC Designs

2.1 MFC Components

An MFC must have an anode and a cathode. The anodic chamber is always kept anaerobic, while the cathodic chamber can be aerobic or anaerobic depending on whether oxygen or another oxidant such as sulfate or nitrate is reduced in the cathodic chamber. MFC reactions can be made without a membrane. However, to achieve a high Coulombic efficiency, the current MFC technology still relies on a membrane [109]. There are many different MFC designs using various materials. Proper selection of reactor configuration and materials is critical to the feasibility for practical MFC applications [230].

2.1.1 Anode

Anodic material is critical to electron transfer efficiency that is an important factor in the overall performance of MFCs [38, 111, 231]. A good anode facilitates the electron transfer donated by an anaerobic biofilm from the oxidation of organic carbons [153, 187]. Due to biofilm catalysis of the anodic reaction, electrocatalytic precious metals such as platinum can be replaced with other inexpensive conductive materials. An ideal anodic material should have excellent conductivity, good chemical stability, high specific surface area including high porosity for

sessile cell coverage, low-cost and non-cytotoxic. They should also be durable, inexpensive and easily made [109, 231]. The anode should be biocompatible for good biofilm attachment [174]. Activation polarization loss is a major contributing factor in reduced MFC performance and often anode material is to blame [38]. Cheap and abundant anode materials are required for large-scale MFC applications because the electron reaction is a surface-based reaction rather than a volume-based reaction as in suspension fermentation. A large overall anode surface area is required to carry out the oxidation of organic carbons with a high throughput. The most commonly used anode materials are carbon-based electrodes such as plain graphite [161], carbon paper [99], carbon-cloth [70], carbon felt [81], reticulated vitreous carbon [57], graphite foam [21], graphite granules [163] and graphite fiber brushes. Logan et al. [107] reported a high power density of 2,400 mW/m^2 using a graphite fiber brush electrode in a small MFC. Corrosion resistant stainless steel has also been used [39].

Research is underway to improve the conductivity and biocompatibility of carbon-based electrodes that are feasible for practical applications. Various metals and metal oxides were used as coating on carbon materials and to enhance the power generation of MFCs [114, 146, 157, 180, 205]. The chemical modification of carbon materials also increased performance of the anode [24, 45, 46, 143, 147, 159, 231, 232]. Among these methods ammonia-treated carbon cloth-anode was the most successful. It boosted power density from 1,640 to 1,970 mW/m^2 [24].

2.1.2 Cathode

The cathodic reaction is another bottleneck in MFC performances [74, 110, 151, 176]. Reaction efficiency is found to be dependent on the concentration and the species of electron acceptor, proton availability, catalyst performance and electrode structure.

Some materials that are used as the anode can also be used as the cathode. Plain graphite/carbon electrode is a commonly used electrode material due to a low-cost. Oxygen is an ideal electron acceptor for cathodic reactions because it has a very high reduction potential. It is also readily available from air and its end product after reduction is water [48, 80, 152, 217]. However, the cathodic reaction of oxygen reduction on a plain graphite/carbon electrode is slow and usually leads to a high overpotential. Platinum is an excellent catalysis for oxygen reduction. Pure platinum cathodes are limited to only lab MFC devices. To minimize the cost, it has been used to modify the cathode with a three- to four-fold increase in current [151]. Platinum use in this fashion is still too expensive. It also suffers from sulfide poisoning [152], especially in large-scale applications such as wastewater treatment. Various other metals and their complexes have been investigated to replace prohibitively expensive platinum for cathodes. Fe(III) [146, 147, 204], cobalt complexes [22, 227], manganese oxide [118, 171] and iron complexes [228] have all been investigated as cathode materials in MFC systems.

Instead of oxygen, ferricyanide can be used as electron acceptor in lab MFC systems for parametric studies [104, 147, 162]. Ferricyanide as catholyte has a low overpotential, leading to a cathode working potential close to its open circuit potential [104]. Permanganate is another electron acceptor that provides power density duo to its higher reduction potential [221]. However, all these non-renewable electron acceptors are not suitable for practical applications because of the need for repeated additions, cost and disposal problems.

In recent years, biocathodes using biofilms to catalyze cathodic reactions have gained considerable traction. The versatility of biocathodes allows the use of not only just oxygen, but also other electron acceptors such as sulfate and nitrate that are common contaminants in agriculture runoffs [191]. Biocathodes can make MFCs more sustainable and less expensive. For example, using biocathodes can avoid sulfur poisoning of expensive platinum electrodes and the need for electron mediators will be eliminated [58, 67].

2.1.3 Proton Exchange System

In a typical MFC, a proton exchange membrane (PEM) is used to partition the anodic and cathodic chambers to avoid direct oxidation of the substrate in the anodic chamber by oxygen [97, 99] that invariably reduces Coulombic efficiency. The maintain electroneutrality in the anodic chamber, for every electron transported to the anode, one proton must migrate through the PEM from the anodic chamber to the cathodic chamber [97, 104, 217]. A good PEM can prevent oxygen diffusion completely, PEM is a significant mass transfer barrier for proton transport. This manifests as a large internal resistance for MFCs. In the absence of convective flow, a large PEM surface area is needed for sufficiently fast proton transfer. This increases costs considerably. Furthermore, membranes are easily fouled, especially if wastewater is used.

Different types of proton exchange devices have been investigated by researchers over the years. They include cation exchange membrane (CEM), anion exchange membrane (AEM), bipolar membrane (BPM), microfiltration membrane (MFM), ultrafiltration membrane (UFM), salt bridge, glass fibres and porous fabrics [97]. In lab-scale MFC devices, Nafion is the most popular PEM because it has a good selectivity for proton permeation [38, 104]. However, it is too costly for large-scale applications.

Researchers have realized that a membrane is not necessarily needed in all MFCs, such as sediment MFCs [168], or specially designed single-chamber MFCs [23, 98]. Single-chamber membrane-less MFCs could achieve a higher power density due to a lower internal resistance. However, the unavoidable partial mixing of oxidants with organic carbons lower the Coulombic efficiency and catalytic activities of electricigens [98, 100].

2.2 Microbial Communities in MFCs

2.2.1 Exoelectrogenenis

Almost all reported MFCs employ bacterial biofilms as biocatalysts, either as a pure culture biofilm or a mixed-culture biofilm consortium. It is possible that a highly electrogenic eukaryotic biofilm such as a fungal biofilm in a pure culture or in a mixed-culture consortium will be discovered in the future. To investigate MFC mechanisms in a laboratory setting, a pure culture biofilm is often used. However, it is almost certain that a biofilm consortium will be used in real world applications such as wastewater treatment, because a mixed culture can process various substrates in a wastewater stream more effectively. A pure culture biofilm in wastewater treatment may be contaminated by native microbes and can be replaced completely over time. The mixed cultures capable of exocellular electron transfer include iron-reducing bacteria [81, 148], glucose and starch fermenting bacteria [21], sulfate-reducing bacteria [33] and others such as *E. coli* [155], *Enterobacter aerogens* and so on. A list of different microorganisms used in anode biofilms is shown in Table 1.

How sessile cells donate electrons to the anode in an MFC is a key issue in MFC mechanisms. Up to now, three electron transfer mechanisms have been mentioned in the literature (Fig. 2): (i) direct electron transfer (DET) [12], (ii) mediated electron transfer (MET) [162], and (iii) electron transfer through conductive pili (i.e., nanowires) [52, 169]. To study each electron transfer mechanism, a pure culture biofilm is preferred over the use of a mixed-culture biofilm.

DET occurs by a directly physical contact between the bacterial cell membrane (i.e., the active sites of extracellular cytochromes of the microorganisms) and the anode surface. The electrons released from the substrate during oxidation can be collected by the anode with the help of extracellular cytochromes. However, the DET reactions have poor electrode kinetics because the active sites are typically buried deep within the cytochromes that are large proteins. Only a monolayer of sessile cells that are in direct contact with the anodic surface are electrochemically active. This means all the other layers of sessile cells are non-productive cells. For this reason, MFCs rely on DET for electron transfer exhibit a low power density. Furthermore, many microorganisms relying on DET are unable to digest complex substrates such as glucose. So far, only *Rhodoferax ferrireducens* [21] has been reported to utilize glucose. Because of their evolutionary history, many microorganisms, especially *Geobacter* and *Shewanella* strains can only utilize relatively simple substrates such as volatile fatty acids and short-chain alcohols [112].

Electron mediators, either endogenic and exogenic, play an essential role in electron transport for microbes (such as *E. coli*, *Pseudomonas*, *Proteus*, and *Bacillus* species) that are unable to transfer the electrons to the anode [112]. The MET reactions are based on dissolved redox species. MFCs utilizing MET often have much better performances than those based on DET. A number of compounds such as phenazines, phenothiazines, phenoxazines and quinines have been

Table 1 Species diversity in anode chamber

No.	Species	References
1	*E.Coil*	[55, 145, 147, 155, 160, 186, 220, 225, 232]
2	*Proteus vulgaris*	[3, 85, 86, 207, 223]
3	*Erwinia dissolvens*	[211]
4	*Pseudomonas aeruginosa*	[55, 162, 163]
5	*Desulfovibrio desulfuricans*	[33, 142, 229]
6	*Shewanella putrefaciens*	[77, 78, 81, 143, 146, 172]
7	*Actinobacillus succinogenes*	[145]
8	*Clostridial sp.*	[148, 156]
9	*Rhodoferax ferrireducens*	[21]
10	*Aeromonas hydrophila*	[150]
11	*Geobacter sulfurreducens*	[12, 131, 169, 170, 210]
12	*Enterococcus faecium*	[162]
13	*Pseudomonas aeruginosa*	[162]
14	*Clostridium butyricum* or *Clostridium beijerinckii*	[132]
15	*Geopsychrobacter electrodiphilus*	[60]
16	*Dessulfobulbus propionicus*	[61]
17	*Klebsiella pneumoniae*	[120, 171]
18	*Geothrix fermentans*	[13]
19	*Geobacter metallireducens*	[122]
20	*Paracoccus denitrificans* and *Paracoccus pantotrophus*	[166]
21	*Saccharomyces cerevisiae*	[28, 195, 214]
22	*Shewanella oneidensis*	[10, 11, 62, 117, 158, 175]
23	*Hansenula anomala*	[157]
24	*Acidiphiliumcryptum*	[14]
25	*Rhodopseudomonas palustris* DX-1	[218]

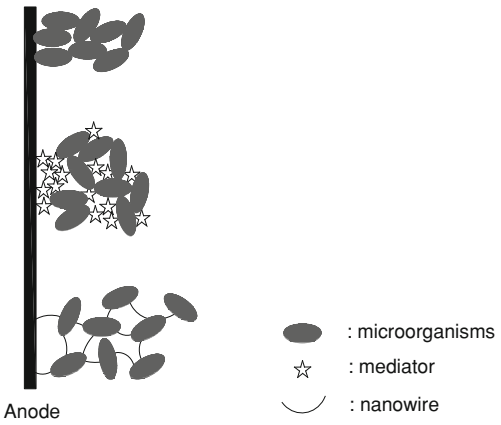

Fig. 2 Electron transfer mechanisms in microbial fuel cells

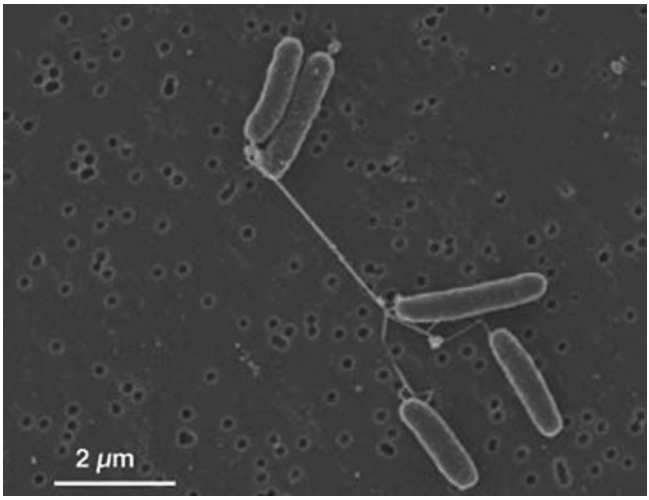

Fig. 3 SEM image of *S. oneidensis* MR-1 showing pili (Reprinted from [53] with permission from PNAS)

investigated as artificial redox mediators [8, 9, 37, 144, 145, 178, 199]. Two types of endogenic redox mediators can be secreted by microbes: (i) organic, reversibly reducible compounds (secondary metabolites); (ii) oxidizable metabolites (primary metabolites) [187]. Externally added electron mediators are unlikely practical because of cost and pollution issues. Locally secreted electron mediators deliver the mediators where they are needed instead of wasting most them in the bulk liquid. In a synergistic biofilm, one type of microbes may deliver mediator to the other type to facilitate electron transfer. This may be one of the reasons that mixed-culture biofilm consortia tend to perform better in MFCs. Selection from naturally occurring biofilms, through mutagenesis, or even genetic engineering will likely create biofilms that will eventually eliminate the charge transfer bottleneck in MFCs.

The DET mechanism usually relies on direct cell wall to electrode contract to transfer electrons. Recent studies indicated that conductive pili (nanowires) enhance electron transfer. "Wired" by nanowires electrons, more than one monolayer of sessile cells can become productive electron donors, thus potentially increasing MFC power density. Nanowires boost the formation of thicker electroactive biofilms, thus relieving charge transfer bottleneck. Such nanowires were discovered in *Geobacter* and *Shewanella* strains [52, 169]. It is possible that in some biofilm consortia, nanowires may exist extensively in biofilm consortia because improved electron transfer can facilitate respiration. The nanowires of *S. oneidensis* MR-1 are shown in Fig. 3. Research is already underway to select or create super bugs with hyperpilation to eliminate charge transfer resistance in bioelectrodes.

2.2.2 Bacteria in Biocathodes

The cathodes of most MFCs are abiotic chemical cathodes. But these abiotic cathodes usually require catalytic electrodes or added electron mediators to remove electrons from the cathode. Such electrodes are expensive and the need for mediators is not feasible for any sustainable MFC operations. Thus they are not practical for real world MFC applications [58, 67]. Recently, biocathodes that use microorganisms to catalyze cathodic reactions have gained popularity among MFC researchers. Many publications have been published in the literature in the last few years. Table 2 shows some electrogenic bacteria used in biocathodes. They included Gram-positive and Gram-negative bacteria, such as *Leptothrix* [134], *Acinetobacter* [50, 165], *Shewanella* [50], *Pseudomonas* [34], *Enterobacter* [34], *Micrococcus* [34], *Chlorella* [154], and Methano-bacterium [26].

Numerous studies have been reported on the electron transfer mechanisms from microbes to the anode in MFCs. However, electron transfer from cathode to microbes in a biocathode has yet been thoroughly investigated by MFC researchers [113]. In bioanodes and biocathodes, electron transfer directions are in the opposite direction. It is reasonable to believe that the electron transport mechanisms involved in anodes are suitable for biocathodes as well, but experimental proof is needed for each mechanism. Both direct and indirect electron transport mechanisms have been mentioned but not fully proven experimentally [67]. Just like electron transfer in anodes, DET in the cathode also requires the direct contact between the monolayer sessile cells at the bottom of the biofilm and the cathodic surface, and the extracellular macromolecules such as cytochromes achieve electrons from the cathode. With DET, only the monolayer sessile cells can transfer electrons. This means that a higher surface density of the monolayer is beneficial, while a thicker biofilm, on the contrary, may actually hinder electron transfer rate by slowing down mass transfer of oxidant from the bulk catholyte solution to the cathodic surface. Behera et al. [7] reported that an increase in the cathode biofilm thickness decreased the power generation of their MFC. With redox active electron mediators or conductive pili, indirect electron transfer can occur between the cathode and the sessile cells. In both cases, more than one layer of sessile cells can transfer electrons, resulting in higher power output due to the faster rate of metabolism.

Nitrate and sulfate are present in agriculture runoffs. Their removal from wastewater is desirable. Table 2 shows that nitrate was used as an oxidant for biocathodes by several research groups because nitrate has a high standard reduction potential quite close to that for oxygen. This results in a higher cell voltage output. Compared with nitrate reduction, sulfate reduction has a much lower reduction potential (−217 m V for SO_4^{2-}/HS^-) [206] and the reduction reaction produces hydrogen sulfide gas that is highly toxic and foul-smelling at very low concentrations. This is probably why researchers have shied away from using sulfate many decades after it was mentioned by Lewis [96] as an oxidant for biocathodes covered with sulfate-reducing bacteria. Sulfate reduction does have unique advantages. Unlike nitrate, sulfate is widely available in many natural

Table 2 Summary of cathode microbes

Biocathode microbe types	Oxidants	Reactor structure	Reference
Enrichment culture	O_2	Two-chamber	[29]
Enrichment culture	O_2	Two-chamber	[49]
Enrichment culture	O_2	Two-chamber	[165]
Acinetobacter calcoaceticus	O_2	Two-chamber	[165]
Sphingobacterium multivorum	O_2	Two-chamber	[165]
Uncultured clone C11r0	O_2	Two-chamber	[165]
Enrichment culture	O_2	Two-chamber	[224]
Enrichment culture	O_2	One-chamber	[2]
Enrichment culture	O_2	Two-chamber	[222]
Acinetobacter calcoaceticus	O_2	Two-chamber	[50]
Shewanella putrefaciens	O_2	Two-chamber	[50]
Pseudomonas aeruginosa	O_2	One-chamber	[34]
Pseudomonas fluorescens	O_2	One-chamber	[34]
Brevundimonas diminuta	O_2	One-chamber	[34]
Burkholderia cepacia	O_2	One-chamber	[34]
Enterobacter cloacae	O_2	One-chamber	[34]
E. coli	O_2	One-chamber	[34]
Shigella flexneri	O_2	One-chamber	[34]
Acinetobacter sp.	O_2	One-chamber	[34]
Kingella kingae	O_2	One-chamber	[34]
Kingella denitrificans	O_2	One-chamber	[34]
Micrococcus luteus	O_2	One-chamber	[34]
Bacillus subtilis	O_2	One-chamber	[34]
Staphylococcus carnosus	O_2	One-chamber	[34]
Enrichment culture	NO_3	Two-chamber	[30]
Enrichment culture	NO_3	Two-chamber	[213]
Enrichment culture	NO_3	Two-chamber	[95]
Dechloromonas sp. and *Azospira sp.*	Perchlorate	Two-chamber	[208]
Enrichment culture	Perchlorate	Two-chamber	[193]
Enrichment culture	Trichloroethene	Two-chamber	[4–6]
Geobacter lovleyi	Tetrachloroethene	Two-chamber	[200]
Enrichment culture	Acetate	Two-chamber	[198]
Geobacter sulfurreducens	Fumarate	One-chamber	[40]
Enrichment culture	CO_2	Two-chamber	[18, 26, 212]
Chlorella vulgaris	CO_2	Two-chamber	[154]
Geobacter sulfurreducens	U(VI)	Two-chamber	[54]
Enrichment culture	Cr(VI)	Two-chamber	[65, 66, 201]

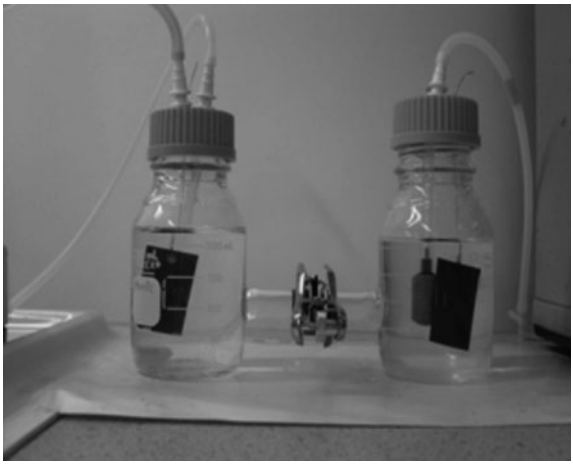

Fig. 4 H-type microbial fuel cells (Reprinted with permission from [105]. Copyright 2006 American Chemical Society.)

environments such as seawater and brackish water. New technologies are available to dispose of hydrogen sulfide gas. For example, the new SWAP process (http://www.swapsol.com/) reacts hydrogen sulfide with CO_2 with heterogeneous catalysis to produce sulfur and arsuls in an exothermic reaction. The CO_2 generated by the anodic chamber during organic carbon oxidation can be directly sequestrated, making this kind of MFCs attractive for carbon credits.

2.3 Basic Architecture

2.3.1 Typical Dual-Chamber MFCs

Different reactor configurations have been designed for MFC system [38]. The classical MFC design has an "H" shape (Fig. 4) with its anodic chamber and cathodic chamber connected by a CEM, or sometimes a salt bridge [104]. A salt bridge, although less expensive, has a high internal resistance. In laboratory research, MFCs are typically run in a batch mode. H-shape MFCs are popular for basic parametric studies of MFCs such as testing different biofilms and substrates, electrodes and membranes. Because of the long distance between the two electrodes, these kinds of MFCs have a high internal resistance.

2.3.2 Typical Single-Chamber MFCs

Researchers have proved that a dedicated cathode chamber to hold a catholyte solution is not necessary when oxygen is used as the terminal electron acceptor. A wetted cathode can be directly exposed to air (Figs. 5 and 6). Park and Zeikus [147]

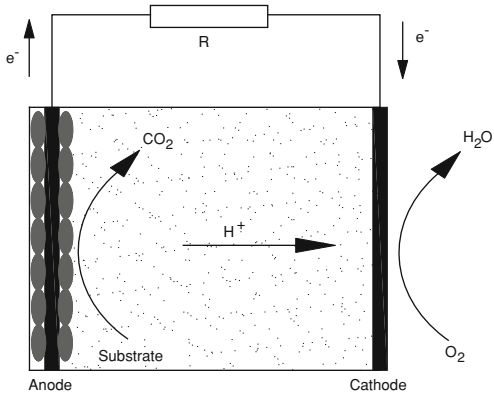

Fig. 5 Schematic of a single-chamber MFC with air-cathode

reported this kind of single-chamber MFC design. This simple design can be more efficient and it is now widely used [98].

Figure 7 shows a simplistic design of an upward flow single-chamber MFC for wastewater treatment. This design is similar to the one used by Jang et al. [72] to treat a modified artificial wastewater. Without a membrane, internal resistance is greatly reduced because proton transport through convective flow is permitted. The convective flow reduces oxygen back diffusion. Upward flow helps oxygen to move upward because gas bubbles move upward. This kind of simple and low-cost design permits high flow rates. Because of the absence of PEM, further improvement in Coulombic efficiency is needed. Figure 8 is an alternative to Fig. 7 design when a non-oxygen oxidant such as nitrate or sulfate is supplied in a supplemental liquid stream. Horizontal or even downward flow can be used to reduce energy input in fluid transport. A nitrate reducer biofilm for the cathode is needed if nitrate is supplied as the oxidant to accept electrons at the biocathode. This kind of low-cost simplistic MFC designs probably have the best chances to succeed in real world wastewater treatment.

2.4 Pilot-Scale MFCs

The Advanced Water Management Center at the University of Queensland built a pilot-scale MFC. It had 12 vertical tubular modules with a height of 3 m and total volume of 1 m^3. It used carbon fiber as anode and cathode. This reactor was tested at the Foster's brewery in Yatala, Queensland, Australia using the brewery's wastewater as the substrate. The output power density was 8 W/m^3, much lower than what is needed to be practical.

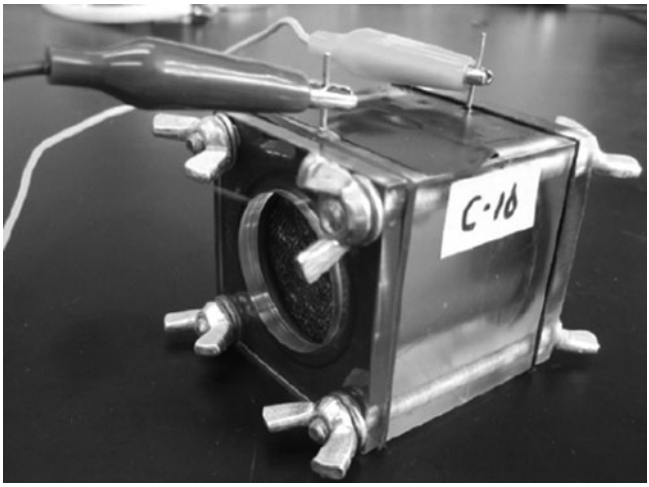

Fig. 6 Image of an actual single-chamber MFC showing an exposed air-cathode (Reprinted from [106] with permission from Elsevier.)

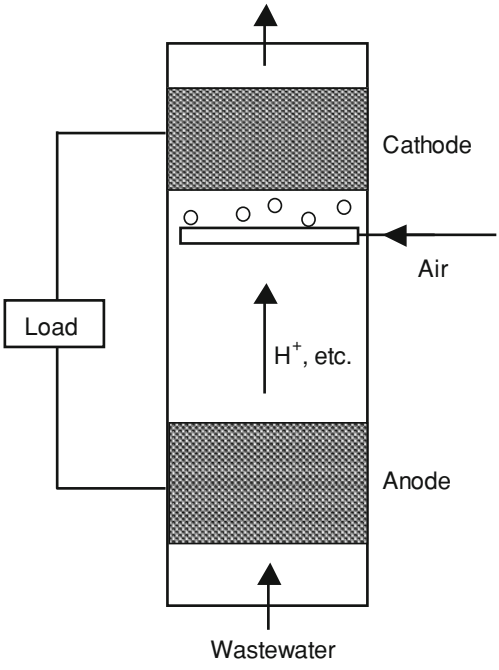

Fig. 7 Simplistic design of membrane-less MFC with upward flow and air-cathode

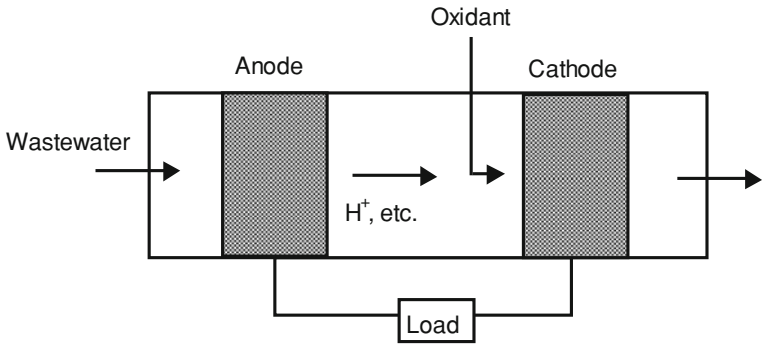

Fig. 8 Simplistic design of a membrane-less MFC with a biocathode for wastewater treatment

3 MFCs for Electricity Generation

3.1 Fundamental

In MFCs, bacteria in the anode chamber are intentionally isolated from dissolved electron acceptors (i.e., oxidants), so the electrons generated from microbial respiration can only transfer to an insoluble acceptor (i.e., the anode). The different electrochemical potentials between the respiratory enzymea and the electron acceptor in the cathodic chamber facilitates electrons transfer from the anode to the cathode. The numbers of transferred protons (via the CEM) and electrons (via the external circuit) are equal so that the system maintains electro-neutrality [106]. Power is harvested from the MFC by a load in the external circuit (Fig. 1).

3.2 Influencing Factors on MFCs Performances

The thermodynamic driving force in an MFC is the difference between the reduction potential of the organic substrate and that of the terminal electron acceptor [164]. The organic substrate has a lower reduction potential and thus it is oxidized. However, the MFC output voltage and current are not determined by the thermodynamic driving force alone because the electrons are transferred to the anode from oxidation of the organic substrate through a complex respiratory chain. Biocatalysis by the biofilm plays a key role in electron transfer. There are many factors that affect MFC performances.

3.2.1 Effect of MFC Reactor Structures

It has been proven experimentally that the improvement of reactor constructions can significantly improve MFC performances. Various designs have emerged to increase power density. They include miniature, cylindrical, upflow, baffled and stacked reactors. Many designs are aimed at reducing Ohmic losses and increasing proton diffusion [217]. Minimization of the distance between the anode and the cathode leads to increased power output. Thus, it is not surprising that the highest reported power density per unit chamber volume came from miniature MFCs [175] in part because of their short proximity between the anode and the cathode. Single-chamber MFCs also have a reduced distance between the anode and the cathode.

3.2.2 Effect of Electrode Materials

Electrode materials, structure, and distance can affect the overall performance of an MFC system. Anode material and structure affect biofilm attachment, electron transfer, and, in some cases, direct substrate oxidation. Meanwhile, cathode materials and structure have an effect on cathodic reaction efficiency. The distance between the anode and the cathode has a direct impact on the internal resistance of MFCs.

A platinumized carbon-cloth anode placed in an *E. coli* culture grown on glucose anaerobically generated a current of 2–4 mA, while no current flow was observed with unmodified carbon-cloth anode [186]. Pt is also a much better catalyst for oxygen reduction than graphite materials in the cathodic chamber. MFCs with a Pt or Pt-coated cathode resulted in a higher power than that with a plain graphite or graphite felt cathode [128, 135]. One important area of MFC research is the investigation of electrode modifications with metals, metal oxides, non-metallic and conductive polymers. In the pursuit of better electrodes from MFCs, cost must be controlled. MFCs are surface-based bioreactors requiring large electrode surfaces and the cost of electrode will quickly increase in practical applications in which large electrode surfaces are essential.

A cloth electrode sandwiched between the anode and the cathode in a single-chamber MFC with an air-cathode had a much-reduced internal resistance. Its power density of 627 W/m^3 was more than 15 times of those reported for air-cathode MFCs using similar electrode materials [42]. Graphite fiber brush anodes in cube air-cathode MFCs reached a maximum power density of 2,400 mW/m^2 because the system internal resistance declined from 31 to 8 kΩ [107]. By halving the distance between the anode and cathode from 4 to 2 cm, the power generation of a single-chamber MFCs increased from 720 to 1,210 mW/m^2 [100].

3.2.3 Effect of Proton Exchange Systems

The types and surface areas of proton exchange systems also affect the power output of the MFCs because they have impacts on the system internal resistance and concentration polarization loss. The membrane surface area is a major factor for MFC power output if the system performance is not anodic electron transfer controlled. Increasing the area decreases internal resistance over a relatively large range [137]. The power density in an MFC with a salt bridge was found much lower than that with a PEM. Min et al. [122] reported a MFC with a salt bridge that generated a power output of only 2.2 mW/m^2 due to 20 kΩ internal resistance compared to that of 1.3 kΩ with a PEM. Kim et al. [82] compared the performances of cation, anion and ultrafiltration membranes in dual-chamber MFCs. They found that the MFC with an anion exchange membrane produced the highest power density of 610 mW/m^2 compared to 514 mW/m^2 with Nafion.

Removing the membrane leads to further reduction of internal resistance. However, without the barrier, oxygen diffusion into the anode chamber can greatly reduce the Coulombic efficiency of a system. Liu and Logan [98] studied the power output of an MFC equipped with an air cathode and carbon electrodes with and without a PEM. Its maximum power density increased from 0.26 to 0.49 W/m^2 when the PEM was removed, while its Coulombic efficiency decreases dramatically from 40–55% to 9–12%. Much work needs to be done to improve the Coulombic efficiencies of membrane-less MFCs.

3.2.4 Effects of Buffer and Electrolyte

A suitable pH maintained by a buffer is important to microbial metabolism [51]. Many buffers have been used in laboratory media, such as phosphate, bicarbonate, 2-[N-morpholino]ethane sulfonate (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and piperazine-N, N'-bis[2-ethanesulfonate] (PIPES). Different buffers have different conductivities that affect Ohmic resistance and power production [130]. Liu et al. [100] demonstrated that high buffer strength used in single-chamber MFCs not only increased the conductivity of the electrolyte but also improved the proton transport. Adding NaCl to the anode chamber also improved MFC power output. The drawback of increasing ionic strength or buffer strength is obviously the extra cost. He et al. [59] indicated that the optimal initial pH for electricity generation from air-cathode MFCs was between eight and ten, and higher or lower pH would decrease microbial activities. The suitable pH for a particular MFC system likely depends on the microbes and substrates involved. In a dual-chamber MFC, a larger pH difference between anolyte and catholyte solutions produced higher current and voltage [71]. You et al. [221] showed that permanganate in the catholyte solution provided much more electrical power than other electrolytes.

3.2.5 Effect of Substrates

In the literature, many substrates have been used tested. They include various chemical compounds in artificial wastewater solutions, real wastewaters and lignocellulosic biomass. Feed parameters such as type, concentration and feed rate all impact MFC performances. The power density generated with acetate was found to be 66% higher than that from an MFC fed with butyrate in a single-chambered MFC [99]. Sharma and Li [192] evaluated acetate, ethanol and glucose as substrates in the concentration range of 0.5–35 mM in single-chamber MFCs and found that glucose achieved the highest power density. Several studies proved that higher feed concentrations led to increased power output [128, 146]. In continuous flow MFCs, hydrodynamic factors influenced biofilm communities [177] and thus MFC performances [126]. Moon et al. [128] reported that the power density increased when the feeding rate increased from 0.15 to 0.65 mL/min, but when the feeding rate reached 1.0 mL/min, the power density decreased to below 0.65 mL/min. Flow effects also influence mass transfer. They will become more important during MFC scaled-up [69].

3.2.6 Other Factors

Other factors have also been investigated by MFC researchers. The effects of temperature on MFC startup and operational performance were reported by several groups [27, 100, 124, 128]. Light was found to affect the microbial community structure and subsequently MFC performances according to Xing et al. [219]. More and Ghangrekar [129] found that the inoculum with ultrasonic pre-treatment enhanced power output and organic matter removal.

3.3 Progress and Outstanding Works

MFCs convert the chemical energy in organic matters that are usually wastes to electrical energy with the aid of biocatalysis from microorganisms. They are becoming increasingly attractive in the pursuit of green and renewable energy. In MFCs, chemical energy is converted into electricity directly without combustion. Thus, a much higher theoretical conversion efficiency (>70%) is possible compared to thermo-conversion of fuels that are limited by the Carnot cycle thermal efficiency [38].

For the past ten years the power production of MFCs has increased 5–6 orders of magnitude [67]. Currently, the reported power output for larger MFCs (>1 liter) is still lower than the goal of 1 kW/m^3, which is considered as the threshold for feasible industrial applications for bioenergy from organic matters [153]. This threshold is several orders of magnitude lower than the power output from conventional chemical fuel cells such as hydrogen and methanol fuel cells. However,

the comparison between MFCs and chemical fuel cells are unfair, unrealistic and also futile, simply because the fuel densities and fuel costs used in these fuel cells are vastly different. In fact, the wastewater used in MFCs most likely has a negative cost, meaning its use (i.e., treatment) is actually subsidized because it costs a lot of money to treat wastewater using existing technologies.

A mediator-less MFCs using *R. ferrireducens* as biocatalyst could convert over 80% of electrons to current when using glucose as substrate [21]. Rabaey et al. [161] reported a higher electron recovery rate up to 89% with a power density of up to 3,600 mW/m^2. A power density of 4,310 mW/m^2 was achieved in a reformative dual-chamber MFCs [162]. Rosenbaum et al. [179] reported an extremely high Coulombic efficiency of 97% for oxidation of formate in an MFC with Pt black catalysis.

The volumetric power density and Coulombic efficiency have been increased quickly in miniature MFCs. A power density of 1.55 kW/m^3 was obtained from a 2.5-mL MFC with a specific cathode surface area of 280 m^2/m^3 [43]. When ferricyanide was added to the catholyte solution, 2.15 kW/m^3 was generated in 0.335-mL reactor with a specific cathode surface area of 1,920 m^2/m^3 [131]. These results broke the conventional conception that mini-MFCs was too small to perform satisfactorily due to amount of substrates and microorganism.

MFCs technology can be used for electricity generation and wastewater treatment simultaneously. Liu and Logan [98] demonstrated that it was possible to produce electricity from domestic wastewater in a MFC, while at the same time accomplishing wastewater treatment. Although, in this study the power density is low (26 mW/m^2). Various types of wastes such as food processing wastewater, swine wastewater, brewery wastewater and corn stover have been used as fuel for electricity production in MFCs. MFC power densities generated from industrial and domestic wastewaters ranged from 4 to 15 W/m^3 in various reports [44, 98, 123, 136].

MFCs can utilize local wastes as substrates for energy generation in situ. For this reason, this technology is more suitable for long-term energy supply in remote or energy-thirsty regions. The mini-MFCs may power implanted medical devices with the substrates supplied by the human body in the future. Thus, a renewable, long-term power source for implantable devices can be achieved [56]. The waste disposal and power supply are two essential problems during long-term space flight. MFCs can solve both of them simultaneously by recycling waste generated onboard to electricity. MFCs can also be a potential technology for on-site, sustainable wastewater treatment in military forward operating bases such as those currently in the remote regions of war-time Afghanistan. The power generated by MFCs during the wastewater treatment offsets the energy cost that is often ten times higher due to the extreme dangers involved in trucking fuel supplies to the bases. MFCs are particularly suitable for this kind of situations.

One practical application based on MFCs technology is the MFCs-type BOD sensor for the purpose of on-site, on-line and real-time monitoring of practical wastewater, where rapid feedback is essential. A variety of mediated-MFC biosensors were developed [119, 149, 199, 207]. However, due to the toxicity of mediators

these biosensors are less desirable. In recent years, mediator-less MFCs were developed for continuous and real-time BOD monitoring [20, 74, 79, 83, 125, 127]. The device showed a long-term operational stability of over 5 years with minimum maintenance. The Coulombic yields were directly proportional to the strength of the wastewater, and the results were in good agreement with BOD_5 determined by conventional means [83]. All the aforementioned MFC-type BOD sensors were based on dual-chamber MFCs with an aqueous cathode. Single-chamber MFCs, with an air cathode, could also be used as a BOD sensor [87, 115]. This type of biosensor had a good correlation between COD concentration and current when it was fed with real wastewater. In addition, a novel biomonitoring system based on an MFC for detecting toxic substances was developed [36, 84]. This system provided an instantaneous response when toxic substances flow into source water. Although this kind of sensors cannot qualitatively analyze the toxic substances, it may be used for early warning.

3.4 Advantages and Challenges

MFCs are considered one of the green energy choices although the technology is not mature yet. MFCs have many potential advantages over traditional methods of generating electricity such as high conversion efficiency, positive environmental impact, mild operating conditions (ambient temperature, near to neutral pH), a wide range of potential fuels, etc.

There are, however, three main bottlenecks that limit the performance of MFCs. First, in the anode chamber, the combination and interaction of different electron transfer mechanisms and multiple redox species lead to a complex electrochemical behavior. Electron transfer from the biofilm to the anode is hampered by anodic overpotentials (e.g., transfer resistances) [90]. Anodic overpotentials including activation overpotentials, Ohmic losses and concentration polarization lower the voltages generated by MFCs and hence decrease the energy efficiencies. Second, the transfer of proton from the anodic chamber to the cathodic chamber is restricted by a membrane, which leads to pH gradient. This slow transfer rate may lower the bioelectrochemical performance and system stability. The membrane also increases the overall internal resistance and the cost of MFCs [97]. It is also prone to fouling. Third, the reduction rate of oxidant on the surface of cathode is slow and leads to a high overpotential. The cathodic overpotentials include activation losses, charge transfer losses and mass transport losses. And this is considered to be the main contributor to overall performance losses in MFC systems [176].

The main drawbacks for practical MFC applications are the low power density and the high cost. Even though considerable improvements have been made in system performance and cost reduction in the past few years, MFCs are still far from practical. Concerted efforts in research are still needed from several academic

disciplines, such as microbiology, electrochemistry, chemical engineering, electrical engineering and materials science.

4 Microbial Electrolysis Cells (MECs)

4.1 Fundamental

Under regular operating conditions, the oxidation of substrates releases electrons and also protons in the anodic chamber. The protons migrate through the membrane that partitions the anode and cathodic chambers to the cathode. The electrons flow to the cathode through an external circuit, where they finally combine with oxygen and protons to form water in typical air-cathode MFCs. If the cathodic chamber is kept anaerobic and a small external voltage is supplied to augment the electrochemical potential achieved by bacterial degradation of organic matters, hydrogen instead of water can be produced in the cathodic chamber. This process is known as a bioelectrochemically assisted microbial reactor (BEAMR) process or the biocatalyzed electrolysis process, and this kind of modified MFCs are called microbial electrolysis cells (MECs) (Fig. 9) [101, 103].

In the process of degrading an organic substrate such as acetate, the biofilm on the anode can generate a potential of approximately ~ -0.3 V (versus a standard hydrogen electrode).

$$\text{Anode: } CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^- \left(E^0 = -0.28\,\text{V}\right) \quad (3)$$

$$\text{Cathode: } 8H^+ + 8e^- \rightarrow 4H_2 \left(E^0 = -0.42\,\text{V}\right) \quad (4)$$

In Eq. 3, the standard (reduction) potential of acetate is less negative than that for H_2 in biological systems, i.e., $-0.28 > -0.42$ V [19]. Thus, CO_2 reduction instead of acetate oxidation is favored. In order to reverse it, an external voltage is needed. The external voltage must be greater than 0.14 V. This voltage is much lower than the theoretical voltage required for water electrolysis $\left(E^0_{H_2O} = 1.23\,\text{V}\right)$ [19, 182]. In practice, due to various overpotentials, the actual external voltage is much greater. Still, MECs provide energy savings in hydrogen production by harvesting energy from organic carbon degradation. If organic carbons with more negative standard potentials such as lactate and formate are used, the energy saving would be more significant.

4.2 Factors that Affect MEC Performances

MFCs and MECs are based on the same bioelectrochemical principles, but there are some differences. Compared to an MFC with air-cathode, an MEC's cathode

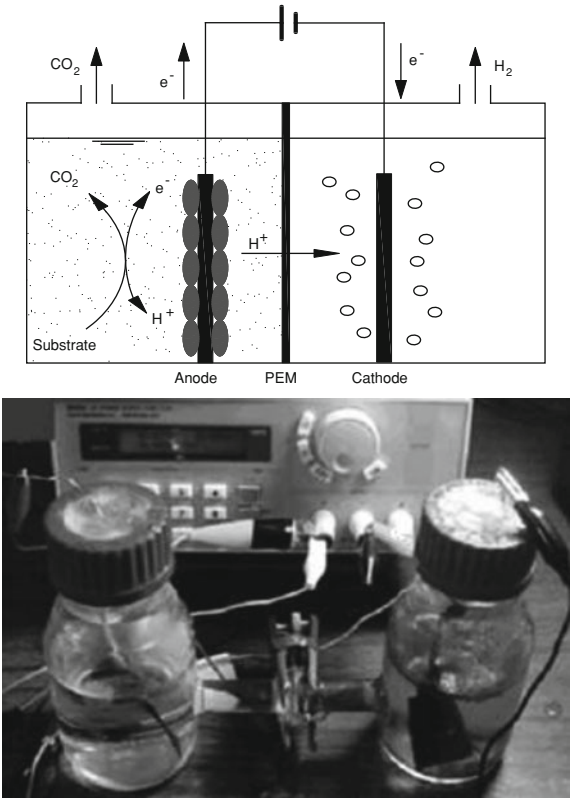

Fig. 9 Schematic of a microbial electrolysis cell (*top*) and a laboratory-scale setup (*bottom*) (Reprinted with permission from Liu et al. [101]. Copyright 2005 American Chemical Society.)

must be strictly anaerobic for hydrogen production. Furthermore, the effect of the external voltage on the biofilm on the anode was not systematically investigated only until recently [19, 216].

4.2.1 Effect of Cell Structures

Because MFCs and MECs can have identical anodic reactions, they can use the same substrate and biofilm in the anodic chamber. However, scale-up and optimization can be different for MECs [63]. Hydrogen production via electrohydrogenesis in MECs was first introduced by Liu et al. [101]. They designed a bottle-type two-chamber MECs, and by using acetate as a substrate. They achieved a yield of 2.9 mol H_2/mol acetate with 0.85 V of external voltage. The Coulombic efficiency and the cathodic conversion efficiency (CCE, i.e., the ratio of e^- equiv donated to H_2 normalized to the e^- equiv transferred in the circuit from the anode to the cathode) were 78 and 92%, respectively [101]. Several other studies reported lower external voltages while achieving similar yields [15, 16, 63]. With the goal

of maximizing the H_2-harvesting efficiency, Lee et al. [93] designed an upflow-type single-chamber MEC by positioning the metal-catalyst-free cathode near the top of the MEC. The CCE from coulombs to H_2 was up to 98 ± 2%, the CE was 60 ± 1% when evaluated in the batch mode with an initial acetate concentration of 10 mM. In comparison, the CCE was only 48% in the bottle-type single-chamber MECs having a cathode alongside the anode, and the CCE dropped to 26% for 100 h of reaction time [91].

4.2.2 Effect of Electrode Materials

Electrode selections are critical in MEC performances and economic feasibilities. Various electrode materials have been used in lab-scale MECs, including carbon felt [91], stainless steel brushes [16], stainless steel and nickel alloys [188], graphite granules [31], graphite rods [140, 141], and graphite fibers [92]. A 2.5 L continuous-flow MEC with multiple electrodes achieved a maximum hydrogen production of 0.53 $m^3/m^3/d$ and an energy efficiency of 144% based on energy in hydrogen gas produced and electricity consumption [167].

Pt is prohibitively expensive for use as the cathode catalyst in practical MECs. Various alternatives have been explored by researchers. Among them, stainless steel showed good promises [16, 226]. Call et al. [16] demonstrated that stainless steel brush cathodes with high surface areas produced hydrogen at rates and efficiencies similar to those using Pt-catalyzed cathodes in single-chamber MECs [16]. Electrodepositing Ni oxide layer on stainless steel cathode, electrodepositing Ni, NiW, or NiMo on gas diffusion cathode, and Ni powder cathode also showed some promises [64, 68, 188, 190]. Pd/Pt cathode catalyst was used in a continuous-flow single-chamber MEC with a hydrogen yield of 2 mol H_2/mol acetate and conversion efficiency of 50% [202]. Rozendel et al. [184] used a mixed culture biocathode for hydrogen production achieving a current density of 1.2 A/m^2, 3.6 times higher than that of a control without a biofilm on the cathode [184]. In a follow-up study, their current density increased to 3.3 A/m^2 at the same conditions [73].

4.2.3 Effect of Proton Exchange Systems

Almost all the reported MECs for hydrogen production were dual-chamber reactors that used a membrane to partition the anodic and cathodic chambers to prevent hydrogen diffusion into the anodic chamber where it may be utilized by bacteria. Due to its small molecular size, some hydrogen can still diffuse through the membranes [182]. MECs suffer from a high Ohmic resistance and a high pH gradient across the membranes. Single-chamber MECs with a CEM or AEM have been reported with potential losses of 0.38 V and 0.26 V associated with CEM and AEM, respectively [183]. Sleutels et al. [196], indicating that AEM was superior to CEM.

Experiments without the membrane showed that it was possible to achieve similar or even higher hydrogen recoveries [15]. One study found that the Ohmic loss and the pH energy loss decreased to 0.005 and 0.072 V, respectively after removing the membrane [93]. Tartakovsky et al. [203] achieved a five-fold increase in the hydrogen production rate by using a continuous-flow membrane-less MEC.

4.2.4 Effect of Substrates

Substrates used for MFCs can also be used in MECs for hydrogen production. Selembo et al. [189] found that P-glycerol produced five times more hydrogen than B-glycerol (2.0 vs. 0.41 $m^3/m^3/d$) with an external voltage of 0.9 V, slightly better than glucose (1.9 $m^3/m^3/d$). Cusick et al. [35] compared winery wastewater and domestic wastewater as substrates for hydrogen production in single-chamber, cubic-shaped MECs. They reported that the rate of hydrogen production from winery wastewater (0.17 $m^3/m^3/d$) was lower than that with domestic waste (0.28 $m^3/m^3/d$). The winery wastewater's performance was similar to cellulose (0.11 $m^3/m^3/d$) and valeric acid (0.14 $m^3/m^3/d$) [25]. Kiely et al. [76] found that potato-processing wastewater produced hydrogen at a rate of 0.74 $m^3/m^3/d$ with an external voltage 0.9 V in their MEC while dairy manure wastewater did not produce any measurable amount of hydrogen.

4.2.5 Effect of Externally Applied Voltage

In the aforementioned discussion, 0.14 V was mentioned as the minimum external voltage for hydrogen production from acetate. To overcome overpotentials due to electron transfer resistance and mass transfer resistance [101, 182] in the MEC, the actual external voltage is significantly higher than 0.14 V, typically in the range of 0.6–1.2 V [15, 16, 25, 93, 203].

In one study using acetate as the sole substrate, Chae et al. [19] found that hydrogen gas started to appear when the external voltage was greater than 0.30 V, while a voltage below 0.2 V produced a negligible amount of hydrogen. Tartakovsky et al. [203] showed that hydrogen output increased with the increase of the external voltage in the range of 0.2–1.2 V. Hydrogen production dropped significantly with lower external voltages in membrane-less MECs because of hydrogen consumption by methanogenic microbes [15, 31, 63, 215]. Using a membrane-less MEC, Call and Logan [15] noticed that the overall hydrogen recovery of 90% with an external voltage 0.6 V dropped to 18% at 0.2 V while the methane concentration increased from 0.9–28%. Selembo et al. [188] also confirmed that a lower voltage produced lower and more variable amounts of hydrogen gas and a higher voltage reduced methane production [188]. However, the overall hydrogen recovery efficiency declined when the voltage was further increased to 1.0 V [19].

4.2.6 Other Factors

If the anolyte solution pH is outside the optimal range, the metabolism of the microbes in the biofilm on an anode can be inhibited. An extreme pH, either high or low, can cause the anodic potential to rise, and this hinders the electron transport from the biofilm to the anode, thus reducing the system performance. Wang et al. [216] found that an extremely low pH irreversibly ruined the biofilm on the anode in 24 h, while the effect of an extreme high pH could be recovered for hydrogen production after the pH switched back to neutral.

Kyazze et al. [88] discussed the effects of catholyte solution pH and temperature on hydrogen production from acetate in a dual-chamber tubular MEC. They found that 30°C was the optimal temperature in the range of 18.5–49.4°C and a lower catholyte solution pH enhanced hydrogen production.

Hydrodynamic force can also influence hydrogen production because it can change the thickness and composition of the biofilm, and mass transfer rate in the anode [1, 209]. Stirring in anodic and cathodic chambers can reduce mass transfer resistance especially when electron transfer is relatively fast. Hydrogen production rate increased by an average of 30% by agitating the anolyte solution that resulted an increased of Reynolds number from $\sim$ 900 to $\sim$ 4,900 [1].

4.3 Progress and Outstanding Works

Although hydrogen production from MECs was proposed a short few years ago, rapid developments have led to hydrogen yields approaching 100% [108]. An overview of the major MEC systems that have been published is summarized in Table 3.

The first MEC was designed by Liu et al. only for "proof of concept". It was a classic H-type reactor with two glass bottles separated by a CEM. Hydrogen gas was collected in the headspace of the cathodic chamber. This H-type reactor is known for its high internal resistance [101]. Cheng and Logan developed a compact reactor system using high-temperature ammonia gas modified three-dimensional graphite granule anode and an AEM. Hydrogen gas was produced at a yield of 2.01–3.95 mol/mol (50–99% of the theoretical maximum) with applied voltages of 0.2–0.8 V using acetic acid as substrate. Using glucose as substrate, hydrogen yield reached 8.55 mol/mol with an applied voltage of 0.6 V [25].

Call and Logan [15] demonstrated that high hydrogen recovery and production rates could be achieved in a membrane-less single-chamber MEC, reducing the cost of the system and allowing for a simpler design. Through this membrane-less system, hydrogen production rates reached a maximum of 3.12 $m^3/m^3/d$ at with an applied voltage of 0.8 V. This development is an important step toward making electrohydrogenesis an economically viable process for hydrogen production from wastewater.

Table 3 Summary of MEC performances

MEC type	Substrate	Applied voltage, (V)	H_2 production, (m^3/m^3d)	References
Single[a]	Acetate	1.0	0.33	[183]
Single[b]	Acetate	1.0	0.31	[183]
Single	Acetate	0.8	0.052	[19]
Single	Acetate	0.8	3.12	[15]
Single	Acetate	0.6	0.53	[63]
Single	Acetate	1.16	0.98	[202]
Single	Acetate	0.6	1.7	[16]
Single	Glucose	0.9	1.9	[188]
Single	Acetate	1.0	0.57	[93]
Single	Acetate	1.0	5.4	[68]
Single	Winery wastewater	0.9	0.17	[35]
Single	Domestic wastewater	0.9	0.28	[35]
Dual	Acetate	0.45	0.37	[101]
Dual	Acetate	0.5	0.02	[182]
Dual	Glucose	0.6	1.23	[25]
Dual	Cellulose	0.6	0.11	[25]
Dual[a]	Acetate	1.0	0.4	[196]
Dual[b]	Acetate	1.0	2.1	[196]

[a] CEM
[b] AEM

Lalaurette et al. [89] used an actual fermentation effluent as the substrate for an MEC and improved significantly the overall hydrogen molar yield. A variety of wastewater including winery wastewater, domestic wastewater and potato wastewater was also fed to MECs for hydrogen production.

4.4 Advantages and Challenges

Although hydrogen production from MECs is only a recent development, it already shows its promise as a new way for renewable and sustainable hydrogen production from organic feed stocks that are wastes. The first advantage of MECs is high H_2 yields. And anode-respiring bacteria can almost completely oxidize substrates into CO_2 and H_2O compared to incomplete conversion in dark fermentation [94]. The second advantage of MECs is the ability to harvest high-purify hydrogen with ease because the cathodic chamber does not involve impurities that require difficult and expensive separations. Other methods such as hydrogen production from organic matters through dark fermentation and biomass gasification produce a mixture of hydrogen, carbon dioxide and other gases such as carbon monoxide and hydrogen sulfide [182]. The third advantage is that the microbes used in the anodic chamber of MECs are capable of degrading a huge

variety of organic matters including lignocellulosic biomass. Lee et al. [94] pointed out that the combination of dark fermentation and MEC could achieve even better hydrogen yields than using an MEC alone for complex substrates.

In addition to the drawback of requiring an external energy input, another challenge for MEC production of hydrogen is the need to improve the production rate in order to offset the capital investment. The highest hydrogen production rate reported so far is 0.23 L H_2/L/h with an external voltage of 1.0 V [68], that is far less than the maximum of 7.9 L H_2/L/h from dark fermentation [102]. MECs can produce large quantities of hydrogen from renewable resources such as biomass and wastewater. Significant advances have been made with respect to the performance only a few years since its introduction. To become a mature hydrogen production technology, however, materials for electrodes and membranes, reactor configurations and anode-respiring bacterial metabolism all need further research [94].

5 Other Bioproducts Produced Using the MFCs Technology

5.1 Hydrogen Peroxide Production

Hydrogen peroxide (H_2O_2) is widely used in various industrial applications, such as chemical syntheses, pulp and paper bleaching and textile bleaching [17]. The present methods (e.g., the anthraquinone oxidation process) for H_2O_2 production are all highly energy-intensive [47]. Therefore, a novel, less energy-intensive method for H_2O_2 production is desired.

Instead of producing hydrogen, MECs can also be used to produce H_2O_2 if oxygen is supplied to the cathodic chamber. An external voltage is required to make the process thermodynamically favorable (Fig. 10). Take acetate for example, the reaction process is as follows.

$$\text{Anode}: CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^- \left(E^0 = -0.28\ \text{V}\right) \quad (5)$$

$$\text{Cathode}: 8H^+ + 4O_2 + 8e^- \rightarrow 4H_2O_2 \left(E^0 = 0.68\ \text{V}\right) \quad (6)$$

The overall reaction is exergonic because the redox reaction has a potential of 0.96 V, which means that H_2O_2 could be produced without an external voltage.

Rozendal et al. demonstrated that an MEC with an external voltage of 0.5 V produced H_2O_2 at a rate of 1.9 ± 0.2 kgH_2O_2/m^3/d from acetate with an overall efficiency of $83.1 \pm 4.8\%$ [185]. The system had a much lower energy input of $\sim$ 0.93 kWh/kg H_2O_2 compared to 4.4–8.9 kWh/kg H_2O_2 produced from conventional electrochemical systems [47]. This makes MECs an attractive option for H_2O_2 production from biomass if the production rate can be substantially increased.

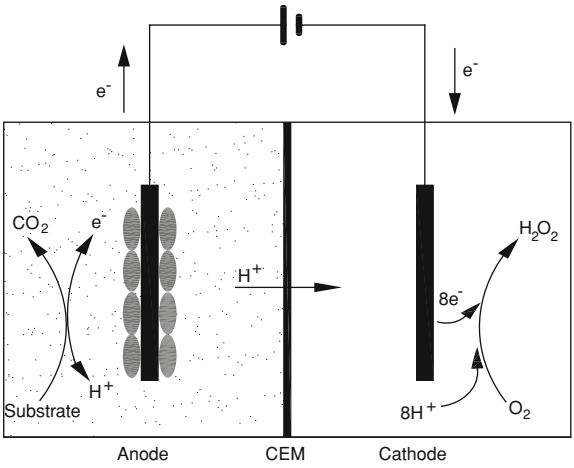

Fig. 10 Working principle of bioelectrochemical H_2O_2 generation

5.2 Methane Production

Renewable biomethane is mainly produced by methanogens in anaerobic methane digesters. With an anodic oxidation of acetate, an external voltage of 0.244 V is needed to make the reduction of CO_2 to methane as shown in Eqs. 7, 8 thermodynamically favorable [26] under standard conditions.

$$\text{Anode} : CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^- \quad (7)$$

$$\text{Cathode} : CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (8)$$

Methanogens are needed for the biocathode to produce methane from inorganic CO_2 anaerobically as shown in Fig. 11. This electrochemical process converts CO_2 into a fuel.

Cheng et al. [26] used a dual-chamber MEC to produce methane from CO_2 with a biocathode covered by an anaerobic biofilm dominated by *Methanobacterium palustre*. They reported an overall efficiency of 80% with the use of acetate as substrate for the anodic chamber. Villano et al. [212] reported an MEC with Coulombic efficiency greater than 80% using a hydrogenophilic methanogenic culture.

MECs may become an alternative to the traditional methane digesters for the conversion of CO_2 to biomethane without the need for expensive precious metal electrodes, if production rate and reactor cost can improve significantly.

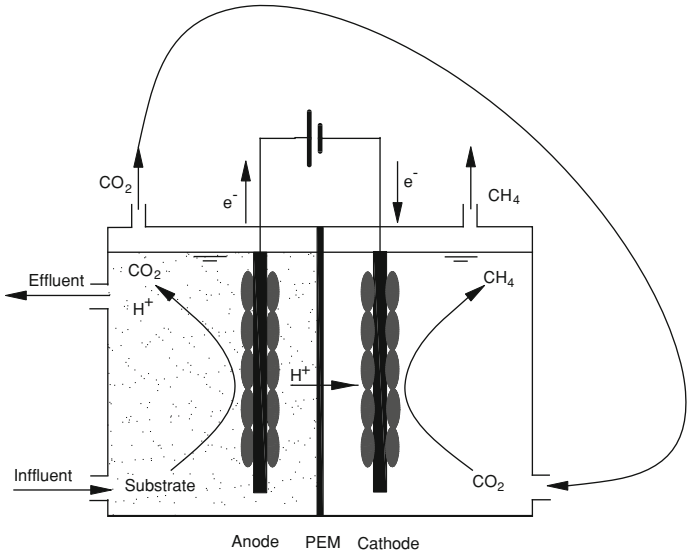

Fig. 11 Schematic for CH_4 production from CO_2 with biocathode. Figure redrawn after Villano et al. [212]

5.3 *Ethanol Production*

Waste biomass can be converted into biogas fuels through mature biological technologies such as anaerobic digestion. Researchers have started investigating electrochemical methods to convert biomass to liquid ethanol fuel [198]. Steinbusch et al. [197] developed a new method that utilizing microbial reduction of volatile fatty acids (VFAs) to produce bioethanol with hydrogen as electron donor. Recently, they investigated the feasibility of using an electrode as the electron donor instead of using hydrogen for biological acetate reduction [198]. The reduction reaction is shown in Eq. 9.

$$CH_3COO^- + 5H^+ + 4e^- \rightarrow CH_3CH_2OH + H_2O \quad (9)$$

In the presence of mixed cultures and methyl viologen that is an electron mediator, when an applied cathode potential was −550 mV, ethanol production started to occur at the cathode in the MEC. Methyl viologen increased ethanol production by six-fold and doubled ethanol concentration compared to the results obtained without the mediator because it inhibited methanogenesis and n-butyrate production. However, ethanol production lasted for only five days because of methyl viologen reacted with the graphite felt biocathode [198].

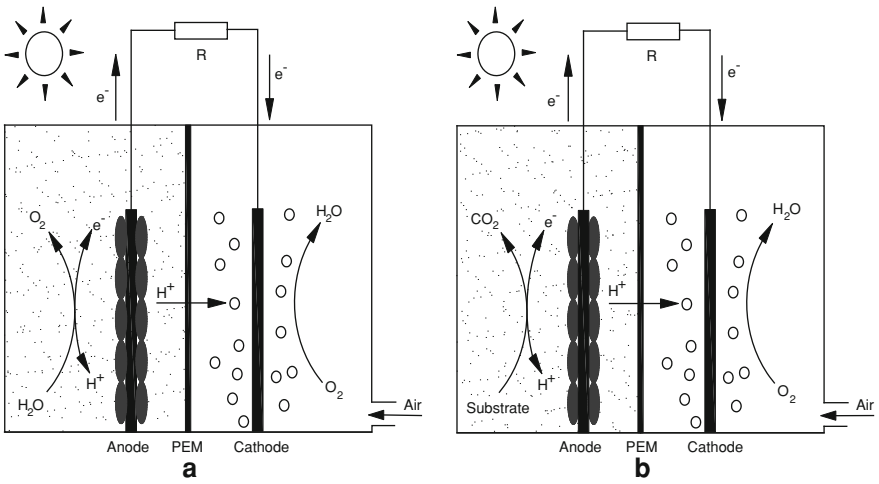

Fig. 12 Two types of photosynthetic MFCs. (Figure redrawn after [181])

5.4 Photosynthetic MFCs

The energy stored in renewable biomass originally came from sunlight. In recent years, some researchers investigated Photosynthetic MFCs (PMFCs) in the pursuit of self-sustaining MFCs. PMFCs are powered by light instead of organic carbons by utilizing phototrophic microorganisms to produce electricity [181, 233]. Two types of PMFCs have been reported in the literature. They are shown in Fig. 12. One type of PMFC relies on photosynthesis as the exclusive energy source, and the other type uses photosynthesis to supplement the energy harvested from the degradation of an organic substrate [181].

The first type of PMFCs will compete directly with photovoltaic cells that are less-costly and more sustainable. Thus, this kind of MFCs will likely remain only as an academic curiosity. The power density of the second type of PMFCs is substantially lower than that of other MFCs [42, 106], although some improvements have been made by using nano-structured electrically conductive polymer polypyrrole [233]. Because this type of PMFCs requires phototrophic microorganisms to digest organic carbons, many highly electrogenic microorganisms that are not phototrophic, but more productive are excluded. A fatal drawback of using light as an energy source in MFCs is that only sessile cells in the anodic biofilm harvest the light energy. This means that the energy from photosynthesis is much less than that from a suspension culture of phototrophic microorganisms.

6 Summary and Perspectives

In view of current energy shortage and grave concerns over global warming due to accelerated carbon emission from fossil fuels, the MFC technology in its various forms represents a promising green technology for renewable and sustainable energy production, especially in a distributed energy system. Although its energy production is small, but its future potential as part of an integrated energy supply system should not be overlooked. Because MFCs use low energy density fuels such as wastewater, power densities from MFCs at best will be three or four orders of magnitude lower than chemical fuel cells that use high density energy sources such as pure hydrogen or methanol. However, MFCs have a huge advantage because they utilize low-grade biomasses that are wastes at no costs. The goal of achieving a stable power output at the level of 1 kW/m^3 is possible through the use of highly electrogenic super bugs that are capable of digesting a multitude of organic substrates from simple VFAs to lignocellulosic biomass. Super bugs can also be engineered to utilize more than one monolayer sessile cells for electron transfer between a biofilm and an electrode because of the presence of locally secreted electron mediators and/or hyperpilation [187]. Super bugs selected from natural biofilm consortia, through mutagenesis or genetic engineering present a true potential game-changer in practical MFC applications.

MFC reactor costs should also be substantially reduced for practically applications. Simplistic designs with low-cost materials for electrodes and other reactor components will likely prevail in the end. When charge transfer limitation is lifted through the use of super-electrogenic microbes, the bottleneck of proton migration from anode to cathode (i.e., electricity flow inside an MFC) will become more pronounced. In order of reduce this internal resistance, membrane-less MFCs will probably be the only practical option, because a PEM does not permit convective flow. It is expensive and easily fouled in wastewater treatment.

MFCs operated as MECs can potentially be used for the production of value-added bioproducts such as hydrogen, methane, hydrogen peroxide and ethanol. Hydrogen, methane and ethanol are also fuels for conventional chemical fuel cells. Any of these chemical fuels produced by MECs can be accumulated and then used to power large devices that cannot be powered by MFCs in situ. Further improvements in production rates and reactor costs are needed for the bioelectrochemical method to be competitive.

References

1. Ajayi FF, Kim KY, Chae KJ et al (2010) Effect of hydrodymamic force and prolonged oxygen exposure on the performance of anodic biofilm in microbial electrolysis cells. Int J Hydrogen Energy 35:3206–3213

2. Aldrovandi A, Marsili E, Stante L et al (2009) Sustainable power production in a membrane-less and mediator-less synthetic wastewater microbial fuel cell. Bioresour Technol 100:3252–3260
3. Allen RM, Bennetto HP (1993) Microbial fuel cells: electricity production from carbohydrates. Appl Biochem Biotechnol 39:27–40
4. Aulenta F, Catervi A, Majone M et al (2007) Electron transfer from a solid-state electrode assisted by methyl viologen sustains efficient microbial reductive dechlorination of TCE. Environ Sci Technol 41:2554–2559
5. Aulenta F, Canosa A, Reale P et al (2009) Microbial reductive dechlorination of trichloroethene to ethene with electrodes serving as electron donors without the external addition of redox mediators. Biotechnol Bioeng 103:85–91
6. Aulenta F, Reale P, Canosa A et al (2010) Characterization of an electro-active biocathode capable of dechlorinating trichloroethene and cis-dichloroethene to ethane. Biosens Bioelectron 25:1796–1802
7. Behera M, Jana PS, Ghangrekar JMM (2010) Performance evaluation of low cost microbial fuel cell fabricated using earthen pot with biotic and abiotic cathode. Bioresour Technol 101:1183–1189
8. Bennetto HP (1990) Electricity generation by microorganisms. Biotechnol Educ 1:163–168
9. Bennetto HP, Stirling JL, Tanaka K et al (1983) Anodic reactions in microbial fuel cells. Biotechnol Bioeng 25:559–568
10. Biffinger JC, Pietron J, Ray R et al (2007) A biofilm enhanced miniature microbial fuel cell using *Shewanella oneidensis* DSP10 and oxygen reduction cathodes. Biosens Bioelectron 22(8):1672–1679
11. Biffinger JC, Pietron J, Bretschger O et al (2008) The influence of acidity on microbial fuel cell containing *Shewanella oneidensis*. Biosens Bioelectron 24(4):906–911
12. Bond DR, Lovley DR (2003) Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl Environ Microbiol 69:1548–1555
13. Bond DR, Lovley DR (2005) Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans*. Appl Environ Microbiol 71(4):2186–2189
14. Borole A, O'Neill H, Tsouris C et al (2008) A microbial fuel cell operating at low pH using the acidophile *acidiphilium cryptum*. Biotechnol Lett 30:1367–1372
15. Call DF, Logan BE (2008) Hydrogen production in a single chamber microbial electrolysis cell lacking a membrane. Environ Sci Technol 42:3401–3406
16. Call DF, Merrill MD, Logan BE (2009) High surface area stainless steel brushes as cathodes in microbial electrolysis cells. Environ Sci Technol 43:2179–2183
17. Campos-Martin JM, Blanco-Brieva G, Fierro JLG (2006) Hydrogen peroxide synthesis: an outlook beyond the anthraquinone process. Angew Chem Int Ed 45:6962–6984
18. Cao X, Huang X, Liang P et al (2009) A completely anoxic microbial fuel cell using a photo-biocathode for cathodic carbon dioxide reduction. Energy Environ Sci 2:441–548
19. Chae KJ, Choi MJ, Lee J et al (2008) Biohydrogen production via biocatalyzed electrolysis in acetate-fed bioelectrochemical cells and microbial community analysis. Int J Hydrog Energy 33:5184–5192
20. Chang IS, Jang JK, Gil GC et al (2004) Continuous determination of biochemical oxygen demand using a microbial fuel cell type biosensor. Biosens Bioelectron 19:607–613
21. Chaudhuri SK, Lovley DR (2003) Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat Biotechnol 21:1229–1232
22. Cheng S, Liu H, Logan BE (2006) Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. Environ Sci Technol 40:364–369
23. Cheng S, Liu H, Logan BE (2006) Increased performance of single-chamber microbial fuel cells using an improved cathode structure. Electrochem Commun 8:489–494
24. Cheng S, Logan BE (2007) Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. Electrochem Commun 9:492–496

25. Cheng S, Logan BE (2007) Sustainable and efficient biohydrogen production via electrohydrogenesis. Proc Natl Acad Sci USA 104:18871–18873
26. Cheng S, Xing DF, Call DF et al (2009) Direct biological conversion of electrical current into methane by electromethanogenesis. Environ Sci Technol 43:3953–3958
27. Cheng S, Xing DF, Logan BE (2011) Electricity generation of single-chamber microbial fuel cells at low temperatures. Biosens Bioelectron 26(5):1913–1917
28. Chiao M, Lam KB, Lin LW (2006) Micromachined microbial and photosynthetic fuel cells. J Micromech Microeng 16(12):2547–2553
29. Clauwaert P, Van Der Ha D, Boon N et al (2007) Open air biocathode enables effective electricity generation with microbial fuel cells. Environ Sci Technol 41:7564–7569
30. Clauwaert P, Rabaey K, Aelterman P et al (2007) Biological denitrification in microbial fuel cells. Environ Sci Technol 41:3354–3360
31. Clauwaert P, Verstraete W (2009) Methanogenesis in membraneless microbial electrolysis cells. Appl Microbiol Biotechnol 82:829–836
32. Cohen B (1931) The bacterial culture as an electrical half-cell. J Bacteriol 21:18–19
33. Cooney MJ, Roschi E, Marison IW et al (1996) Physiologic studies with the sulfate-reducing bacterium *Desulfovibrio desulfuricans*: evaluation for use in a biofuel cell. Enzyme Microb Technol 18(5):358–365
34. Cournet A, Délia M-L, Bergel A et al (2010) Electrochemical reduction of oxygen catalyzed by a wide range of bacteria including Gram-positive. Electrochem Commun 12:505–508
35. Cusick RD, Kiely PD, Logan BE (2010) A monetary comparison of energy recovered from microbial fuel cells and microbial electrolysis cells fed winery or domestic wastewaters. Int J Hydrogen Energy 35:8855–8861
36. Dávila D, Esquivel JP, Sabaté M et al (2011) Silicon-based microfabricated microbial fuel cell toxicity sensor. Biosens Bioelectron 26(5):2426–2430
37. Delaney GM, Bennetto HP, Mason JR et al (1984) Electron-transfer coupling in microbial fuel cells. 2. performance of fuel cells containing selected microorganism—mediator—substrate combinations. J Chem Technol Biotechnol 34B:13–27
38. Du ZW, Li HR, Gu TY (2007) A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Adv 25(5):464–482
39. Dumas C, Mollica A, Féron D et al (2007) Marine microbial fuel cell: use of stainless steel electrodes as anode and cathode materials. Electrochim Acta 53(2):468–473
40. Dumas C, Basseguy R, Bergel A (2008) Microbial electrocatalysis with geobacter sulfur reducens biofilm on stainless steel cathodes. Electrochim Acta 53:2494–2500
41. Fakhru'l-Razi A, Pendashteh A, Abdullah LC et al (2009) Review of technologies for oil and gas produced water treatment. J Hazard Mater 170(2–3):530–551
42. Fan Y, Hu H, Liu H (2007) Enhanced columbic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration. J Power Sources 171:348–354
43. Fan Y, Hu H, Liu H (2007) Sustainable power generation in microbial fuel cells using bicarbonate buffer and proton transfer mechanisms. Environ Sci Technol 41(23):8154–8158
44. Feng YJ, Wang X, Logan BE et al (2008) Brewery wastewater treatment using air-cathode microbial fuel cells. Appl Microbiol Biotechnol 78:873–880
45. Feng CH, Ma L, Li F et al (2010) A polypyrrole/anthraqu- inone-2, 6-disulphonic disodium salt (PPy/AQDS)- modified anode to improve performance of microbial fuel cells. Biosens Bioelectron 25:1516–1520
46. Feng YJ, Yang Q, Wang X et al (2010) Treatment of carbon fiber brush anodes for improving power generation in air-cathode microbial fuel cell. J Power Source 195:1841–1844
47. Foller PC, Bombard RT (1995) Processes for the production of mixtures of caustic soda and hydrogen peroxide via the reduction of oxygen. J Appl Electrochem 25:613–627
48. Franks AE, Nevin KP (2010) Microbial fuel cells, a current review. Energies 3:899–919
49. Freguia S, Rabaey K, Yuan Z et al (2008) Sequential anode-cathode configuration improves cathodic oxygen reduction and effluent quality of microbial fuel cells. Water Res 42:1387–1396
50. Freguia S, Tsujimura S, Kano K (2010) Electron transfer pathways in microbial oxygen biocathodes. Electronchim Acta 55:813–818

51. Gil GC, Chang IS, Kim BH et al (2003) Operational parameters affecting the performance of a mediator-less microbial fuel cell. Biosens Bioelectron 18:327–334
52. Gorby YA, Beveridge TJ (2005) Composition, reactivity and regulation of extracellularmetal-reducing structures (nanowires) produced by dissimilatory metal reducing bacteria. Presented at DOE/NABIR meeting, Warrenton, 20 April 2005
53. Gorby YA, Yanina S, McLean JS et al (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. PNAS 103: 11358–11363
54. Gregory KB, Lovley DR (2005) Remediation and recovery of uranium from contaminated subsurface environments with electrodes. Environ Sci Technol 39:8943–8947
55. Habermann W, Pommer EH (1991) Biological fuel cells with sulphide storage capacity. Appl Microbiol Biotechnol 35(1):128–133
56. Han YF, Yu CL, Liu H (2010) A microbial fuel cell as power supply for implantable medical devices. Biosens Bioelectron 25:2156–2160
57. He Z, Minteer SD, Angenent LT (2005) Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environ Sci Technol 39:5262–5267
58. He Z, Angenent LT (2006) Application of bacterial biocathodes in microbial fuel cells. Electroanalysis 18:2009–2015
59. He Z, Huang YL, Manohar AK et al (2008) Effect of electrolyte pH on the rate of the anodic and cathodic reactions in an air-cathode microbial fuel cell. Bioelectrochem 74:78–82
60. Holmes DE, Nicoll JS, Bond DR et al (2004) Potential role of a novel psychrotolerant member of the family *Geobacteraceae Geopsychrobacter electrodiphilus,* gen. nov., sp. nov., in electricity production by a marine sediment fuel cell. Appl Environ Microbiol 70(10):6023–6030
61. Holmes DE, Bond DR, Lovley DR (2004) Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes. Appl Environ Microbiol 70(2):1234–1237
62. Hou HJ, Li L, Cho Y et al (2009) Microfabricated microbial fuel cell arrays reveal electrochemically active microbes. Plos One 4(8):e6570
63. Hu HQ, Fan YZ, Liu H (2008) Hydrogen production using single-chamber membrane-free microbial electrolysis cells. Water Res 42(15):4172–4178
64. Hu HQ, Fan YZ, Liu H (2009) Hydrogen production in single-chamber tubular microbial electrolysis cells using non-precious-metal catalysts. Int J Hydrog Energy 34:8535–8542
65. Huang LP, Chen JW, Quan X et al (2010) Enhancement of hexavalent chromium reduction and electricity production from a biocathode microbial fuel cell. Bioproc Biosyst Eng 33:937–945
66. Huang LP, Chai XL, Cheng S et al (2011) Evaluation of carbon-based materials in tubular biocathode microbial fuel cells in terms of hexavalent chromium reduction and electricity generation. Chem Eng J 166(2):652–661
67. Huang LP, Regan JM, Quan X (2011) Electron transfer mechanisms, new applications, and performance of biocathode microbial fuel cells. Bioresour Technol 102(1):316–323
68. Hrapovic S, Manuel MF, Luong JHT et al (2010) Electrodeposition of nickel particles on a gas diffusion cathode for hydrogen production in a microbial electrolysis cell. Int J Hydrog Energy 35:7313–7320
69. Ieropoulos I, Winfield J, Greenman J (2010) Effects of flow-rate, inoculum and time on the internal resistance of microbial fuel cells. Bioresour Technol 101:3520–3525
70. Ishii S, Watanabe K, Yabuki S et al (2008) Comparison of electrode reduction activities of *Geobacter sulfurreducen* and an enriched consortium in an air-cathode microbial fuel cell. Appl Environ Microbio 74:7348–7355
71. Jadhav GS, Ghangrekar MM (2009) Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresour Technol 100:717–723
72. Jang JK, Pham TH, Chang IS et al (2004) Construction and operation of a novel mediator- and membraneless microbial fuel cell. Process Biochem 39:1007–1012

73. Jeremiasse AW, Hamelers HVM, Buisman CJN (2010) Microbial electrolysis cell with a microbial biocathode. Bioelectrochem 78:39–43
74. Kang KH, Jang JK, Pham TH et al (2003) A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor. Biotechnol Lett 25(16):1357–1361
75. Karim GW (2008) The world energy crisis-Part 2: Some more vacuum-based solutions. Vacuum 82(6):679
76. Kiely PD, Cusick RD, Call DF et al (2011) Anode microbial communities produced by changing from microbial fuel cell to microbial electrolysis cell operation using two different wastewaters. Bioresour Technol 102:388–394
77. Kim BH, Kim HJ, Hyun MS et al (1999) Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*. J Microbiol Biotechnol 9:127–131
78. Kim BH, Iketa T, Park HS et al (1999) Electrochemical activity of an Fe(III)-reducing bacterium, *Shewanella putrefaciens* IR-1, in the presence of alternative electron acceptors. Biotechnol Tech 13:475–478
79. Kim BH, Chang IS, Gil GC et al (2003) Novel BOD sensor using mediator-less microbial fuel cell. Biotechnol Lett 25:541–545
80. Kim BH, Chang IS, Gadd GM (2007) Challenges in microbial fuel cell development and operation. Appl Microbiol Biotechnol 76:485–494
81. Kim HJ, Park HS, Hyun MS et al (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzyme Microb Technol 30(2):145–152
82. Kim JR, Cheng S, Oh SE et al (2007) Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells. Environ Sci Technol 41:1004–1009
83. Kim M, Youn SM, Shin SH et al (2003) Practical field application of a novel BOD monitoring system. J Environ Monit 5:640–643
84. Kim M, Hyun MS, Gadd GM et al (2007) A novel biomonitoring system using microbial fuel cells. J Environ Monit 9:1323–1328
85. Kim N, Choi Y, Jung S et al (2000) Development of microbial fuel cells using *Proteus vulgaris*. Bull Korean Chem Soc 21(1):44–48
86. Kim N, Choi Y, Jung S et al (2000) Effect of initial carbon sources on the performance of microbial fuel cells containing *Proteus vulgaris*. Biotechnol Bioeng 70(1):109–114
87. Kumlanghan A, Liu J, Thavarungkul P et al (2007) Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter. Biosens Bioelectron 22:2939–2944
88. Kyazze G, Popov A, Dinsdale R et al (2010) Influence of catholyte pH and temperature on hydrogen production from acetate using a two chamber concentric tubular microbial electrolysis cell. Int J Hydrogen Energy 35:7716–7722
89. Lalaurette E, Thammannagowda S, Mohagheghi A et al (2009) Hydrogen production from cellulose in a two-stage process combining fermentation and electrohydrogenesis. Int J Hydrogen Energy 34:6201–6210
90. Larminie J, Dicks A (2000) Fuel cell systems explained. Wiley, New Jersey
91. Lee HS, Torres CI, Parameswaran P et al (2009) Fate of H_2 in an upflow single-chamber microbial electrolysis cell using a metal-catalyst-free cathode. Environ Sci Technol 43(20): 7971–7976
92. Lee HS, Rittman BE (2010) Significance of biological hydrogen oxidation in a continuous single-chamber microbial electrolysis cell. Environ Sci Technol 44:948–954
93. Lee HS, Rittmann BE (2010) Characterization of energy losses in an upflow single-chamber microbial electrolysis cell. Int J Hydrog Energy 35:920–927
94. Lee HS, Vermaas WFJ, Rittmann BE (2010) Biological hydrogen production: prospects and challenges. Trends Biotechnol 28:262–271
95. Lefebvre O, Al-Mamun A, Ng HY (2008) A microbial fuel cell equipped with a biocathode for organic removal and denitrification. Water Sci Technol 58:881–885
96. Lewis K (1966) Symposium on bioelectrochemistry of microorganisms IV. Biochemical fuel cells. Bacteriol Rev 30:101–113
97. Li WW, Sheng GP, Liu XW et al (2011) Recent advances in the separators for microbial fuel cells. Bioresour Technol 102(1):244–252

98. Liu H, Logan BE (2004) Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ Sci Technol 38:4040–4046
99. Liu H, Cheng S, Logan BE (2005) Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ Sci Technol 39:658–662
100. Liu H, Cheng S, Logan BE (2005) Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. Environ Sci Technol 39:5488–5493
101. Liu H, Grot S, Logan BE (2005) Electrochemically assisted microbial production of hydrogen from acetate. Environ Sci Technol 39:4317–4320
102. Lo YC, Lee KS, Lin PJ et al (2009) Bioreactors configured with distributors and carriers enhance the performance of continuous dark hydrogen fermentation. Bioresour Technol 100:4381–4387
103. Logan BE, Grot S (2005) A bioelectrochemically assisted microbial reactor (BEAMR) that generates hydrogen gas. U.S. Patent Application 60/588, 022
104. Logan BE, Hamelers B, Rozendal R et al (2006) Microbial fuel cells: methodology and technology. Environ Sci Technol 40:5181–5192
105. Logan BE, Regan JM (2006) Microbial fuel cells-challenges and applications. Environ Sci Technol 40:5172–5180
106. Logan BE, Regan JM (2006) Electricity-producing bacterial communities in microbial fuel cells. Trends microbial 14(12):512–518
107. Logan BE, Cheng S, Watson V et al (2007) Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environ Sci Technol 41:3341–3346
108. Logan BE, Call D, Cheng S et al (2008) Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environ Sci Technol 42:8630–8640
109. Logan BE (2009) Microbial fuel cells. Wiley, New Jersey
110. Logan BE (2009) Exoelectrogenic bacteria that power microbial fuel cells. Nat Rev Microbiol 7:375–381
111. Lovley DR (2006) Microbial fuel cells: novel microbial physiologies and engineering approaches. Curr Opin Biotechnol 17:327–332
112. Lovley DR (2006) Bug juice: harvesting electricity with microorganisms. Nat Rev Microbiol 4:497–508
113. Lovley DR (2008) The microbe electric: conversion of organic matter to electricity. Curr Opin Biotechnol 19(6):564–571
114. Lowy DA, Tender LM, Zeikus JG et al (2006) Harvesting energy from the marine sediment water interface II: kinetic activity of anodematerials. Biosens Bioelectron 21(11):2058–2063
115. Lorenzo MD, Curtis TP, Head IM et al (2009) A single-chamber microbial fuel cell as a biosensor for wastewaters. Water Res 43:3145–3154
116. Makarieva AM, Gorshkov VG, Li BL (2008) Energy budget of the biosphere and civilization: rethinking environmental security of global renewable and non-renewable resources. Ecol Complex 5(4):281–288
117. Manohar AK, Bretschger O, Nealson KH et al (2008) The use of electrochemical impedance spectroscopy (EIS) in the evaluation of the electrochemical properties of a microbial fuel cell. Bioelectrochem 72(2):149–154
118. Mao L, Zhang D, Sotomura T et al (2003) Mechanistic study of the reduction of oxygen in air electrode with manganese oxides as electrocatalysts. Electrochim Acta 48:1015–1021
119. Matsunaga T, Karube I, Suzuki S (1980) A specific microbial sensor for formic acid. J Appl Microbiol Biotechnol 10:235–243
120. Menicucci J, Beyenal H, Marsili E et al (2006) Procedure for determining maximum sustainable power generated by microbial fuel cells. Environ Sci Technol 40(3):1062–1068
121. Midilli A, Dincer I (2008) Hydrogen as a renewable and sustainable solution in reducing global fossil fuel consumption. Int J Hydrogen Energy 33(16):4209–4222
122. Min B, Cheng S, Logan BE (2005) Electricity generation using membrane and salt bridge microbial fuel cells. Water Res 39(9):1675–1686

123. Min B, Kim JR, Oh SE et al (2005) Electricity generation from swine wastewater using microbial fuel cells. Water Res 39:4961–4968
124. Min B, Roman OB, Angelidaki I (2008) Importance of temperature and anodic medium composition on microbial fuel cell (MFC) performance. Biotechnol Lett 30(7):1213–1218
125. Moon H, Chang IS, Kang KH et al (2004) Improving the dynamic response of a mediator-less microbial fuel cell as a biochemical oxygen demand (BOD) sensor. Biotechnol Lett 26:1717–1721
126. Moon H, Chang IS, Jang JK et al (2005) Residence time distribution in microbial fuel cell and its influence on COD removal with electricity generation. Biochem Eng J 27:59–65
127. Moon H, Chang IS, Jang JK et al (2005) On-line monitoring of low biochemical oxygen demand through continuous operation of a mediator-less microbial fuel cell. J Microbiol Biotechnol 15:192–196
128. Moon H, Chang IS, Kim BH (2006) Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. Bioresour Technol 97:621–627
129. More TT, Ghangrekar MM (2010) Improving performance of microbial fuel cell with ultrasonication pre-treatment of mixed anaerobic inoculum sludge. Bioresour Technol 101: 562–567
130. Nam JY, Kim HW, Lim KH et al (2010) Variation of power generation at different buffer types and conductivities in single chamber microbial fuel cells. Biosens Bioelectron 25:1155–1159
131. Nevin KP, Richter H, Covalla SF et al (2008) Power output and columbic efficiencies from biofilms of *Geobacter sulfurreducens* comparable to mixed community microbial fuel cells. Environ Microbiol 10:2505–2514
132. Niessen J, Schröder U, Scholz F (2004) Exploiting complex carbohydrates for microbial electricity generation-a bacterial fuel cell operating on starch. Electrochem Commun 6(9):955–958
133. Nigel WA (1999) Climate change and global water resources. Global Environ Change 9(1):31–49
134. Nguyen TA, Lu Y, Yang X et al (2007) Carbon and steel surfaces modified by *Leptothrix discophora* SP-6: characterization and implications. Environ Sci Technol 41:7987–7996
135. Oh SE, Min B, Logan BE (2004) Cathode performance as a factor in electricity generation in microbial fuel cells. Environ Sci Technol 38:4900–4944
136. Oh SE, Logan BE (2005) Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. Water Res 39: 4673–4682
137. Oh SE, Logan BE (2006) Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. Appl Microbiol Biotechnol 70:162–169
138. Osman MH, Shah AA, Walsh FC (2010) Recent progress and continuing challenges in bio-fuel cells. Part II. Microbial Biosens Bioelectron 26(3):953–963
139. Pant D, Van Bogaert G, Diels L et al (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 101:1533–1543
140. Parameswaran P, Torres CI, Lee HS et al (2009) Syntrophic interactions among anode respiring bacteria (ARB) and non-ARB in a biofilm anode: electron balances. Biotechnol Bioeng 103:513–523
141. Parameswaran P, Zhang H, Torres CI et al (2010) Microbial community structure in a biofilm anode fed with a fermensubstrate: the significance of hydrogen scavengers. Biotechnol Bioeng 105(1):69–78
142. Park DH, Kim BH, Moore B et al (1997) Electrode reaction of *Desulfovibrio desulfuricans* modified with organic conductive compounds. Biotechnol Tech 11:145–148
143. Park DH, Kim SK (2000) Electricity production in biofuel cell using modified graphite electrode with neutral red. Biotechnol Lett 22:1301–1304
144. Park DR, Zeikus JG (1999) Utilization of electrically reduced Neutral Red by *Actinobacillus succinogenes*: physiological function of Neutral Red in membrane-driven fumarate reduction and energy conservation. J Bacteriol 181:2403–2410

145. Park DH, Zeikus JG (2000) Electricity generation in microbial fuel cells using Neutral Red as an electronophore. Appl Environ Microbiol 66(4):1292–1297
146. Park DH, Zeikus JG (2002) Impact of electrode composition on electricity generation in a single-compartment fuel cell using *Shewanella putrefucians*. Appl Microbiol Biotechnol 59(1):58–61
147. Park DH, Zeikus JG (2003) Improved fuel cell and electrode designs for producing electricity from microbial degradation. Biotechnol Bioeng 81:348–355
148. Park HS, Kim BH, Kim HS et al (2001) A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Clostridium butyricum* isolated from a microbial fuel cell. Anaerobe 7(6):297–306
149. Pasco N, Baronian K, Jeffries C et al (2004) MICREDOX®—development of a ferricyanide-mediated rapid biochemical oxygen demand method using an immobilized *Proteus vulgaris* biocomponent. Biosens Bioelectron 20:524–532
150. Pham CA, Jung SJ, Phung NT et al (2003) A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. FEMS Microbiol Lett 223:129–134
151. Pham TH, Jang JK, Chang IS et al (2004) Improvement of cathode reaction of a mediatorless microbial fuel cell. J Microbiol Biotechnol 14:324–329
152. Pham TH, Rabaey K, Aelterman P et al (2006) Microbial fuel cells in relation to conventional anaerobic digestion technology. Eng Life Sci 6(3):285–292
153. Pham TH, Aelterman P, Verstraete W (2009) Bioanode performance in bioelectrochemical systems: recent improvements and prospects. Trends Biotechnol 27(3):168–178
154. Powell EE, Mapiour ML, Evitts RW et al (2009) Growth kinetics of *Chlorella vulgaris* and its use as a cathodic half cell. Bioresour Technol 100:269–274
155. Potter MC (1911) Electrical effects accompanying the decomposition of organic compounds. Proc R Soc Lond B Biol Sci 84:160–276
156. Prasad D, Sivaram TK, Berchmans S et al (2006) Microbial fuel cell constructed with a micro-organism isolated from sugar industry effluent. J Power Sources 160:991–996
157. Prasad D, Arun S, Murugesan A et al (2007) Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell. Biosens Bioelectron 22:2604–2610
158. Qian F, Baum M, Gu Q et al (2009) A 1.5 μL microbial fuel cell for on-chip bioelectricity generation. Lab on a Chip 9(21):3076–3081
159. Qiao Y, Li CM, Bao SJ et al (2007) Carbon nanotube/polyaniline composite as anode material for microbial fuel cells. J Power Sources 170:79–84
160. Qiao Y, Li CM, Bao SJ et al (2008) Direct electrochemistry and electrocatalytic mechanism of evolved *Escherichia coli* cells in microbial fuel cells. Chem Commun 21(11):1290–1292
161. Rabaey K, Lissens G, Siciliano SD et al (2003) A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol Lett 25:1531–1535
162. Rabaey K, Boon N, Siciliano SD et al (2004) Biofuel cells select for microbial consortia that self-mediate electron transfer. Appl Environ Microbiol 70:5373–5382
163. Rabaey K, Clauwaert P, Aelterman P et al (2005) Tubular microbial fuel cells for efficient electricity generation. Environ Sci Technol 39(20):8077–8082
164. Rabaey K, Verstraete W (2005) Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol 23(6):291–298
165. Rabaey K, Read ST, Clauwaert P et al (2008) Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cells. ISME J 2:519–527
166. Rabaey K, Sompel KVD, Maignien L et al (2006) Microbial fuel cells for sulfide removal. Environ Sci Technol 40(17):5218–5224
167. Rader GK, Logan BE (2010) Multi-electrode continuous flow microbial electrolysis cell for biogas production from acetate. Int J Hydrogen Energy 35:8848–8854
168. Reimers CE, Tender LM, Fertig S et al (2001) Harvesting energy from the marine sediment water interface. Environ Sci Technol 35:192–195
169. Reguera G, MaCarthy KD, Mehta T et al (2005) Extracellular electron transfer via microbialnanowires. Nature 435(7045):1098–1101

170. Reguera G, Nevin KP, Nicoll JS et al (2006) Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. Appl Environ Microbiol 72(11): 7345–7348
171. Rhoads A, Beyenal H, Lewandowski Z (2005) Microbial fuel cell using anaerobic respiration asan anodic reaction and biomineralized manganese as a cathodic reactant. Environ Sci Technol 39:4666–4671
172. Richter H, McCarthy K, Nevin KP et al (2008) Electricity generation by *Geobacter sulfurreducens* attached to gold electrodes. Langmuir 24(8):4376–4379
173. Rifkin J (2002) The hydrogen economy. Penguin Putnam, New York
174. Rinaldi A, Mecheri B, Garavaglia V et al (2008) Engineering materials and biology to boost performance of microbial fuel cells: a critical review. Energy Environ Sci 1:417–429
175. Ringeisen BR, Henderson E, Wu PK et al (2006) High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10. Environ Sci Technol 40(8):2629–2634
176. Rismani-Yazdi H, Carver SM, Christy AD et al (2008) Cathodic limitations in microbial fuel cells: An overview. J Power Sources 180:683–694
177. Rochex A, Godon JJ, Bernet N et al (2008) Role of shear stress on composition, diversity and dynamics of biofilm bacterial communities. Water Res 42:4915–4922
178. Roller SD, Bennetto HP, Delaney GM et al (1984) Electron-transfer coupling in microbial fuel cells: 1 comparison of redox-mediator reduction rates and respiratory rates of bacteria. J Chem Technol Biotechnol 34B:3–12
179. Rosenbaum M, Schröder U, Scholz F (2006) Investigation of the electrocatalytic oxidation of formate and ethanol at platinum black under microbial fuel cell conditions. J Solid State Electrochem 10:872–878
180. Rosenbaum M, Zhao F, Quaas M et al (2007) Evaluation of catalytic properties of tungsten carbide for the anode of microbial fuel cells. Appl Catal B Environ 74:261–269
181. Rosenbaum M, Schröder U (2010) Photomicrobial solar and fuel cells. Electroanalysis 22:844–855
182. Rozendal RA, Hamelers HVM, Euverink GJW et al (2006) Principle and perspectives of hydrogen production through biocatalyzed electrolysis. Int J Hydrogen Energy 31(12): 1632–1640
183. Rozendal RA, Hamelers HVM, Molenkamp RJ et al (2007) Performance of single chamber biocatalyzed electrolysis with different types of ion exchange membrances. Water Res 41(9):1984–1994
184. Rozendal RA, Jeremiasse AW, Hamelers HVM et al (2008) Hydrogen production with a microbial biocathode. Environ Sci Technol 42:629–634
185. Rozendal RA, Leone E, Keller J et al (2009) Efficient hydrogen peroxide generation from organic matter in a bioelectrochemical system. Electrochem Commun 11(9):1752–1755
186. Schröder U, Niessen J, Scholz F (2003) A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. Angew Chem 115:2986–2989
187. Schröder U (2007) Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. Phys Chem Chem Phys 9:2619–2629
188. Selembo PA, Merrill MD, Logan BE (2009) The use of stainless steel and nickel alloys as low-cost cathodes in microbial electrolysis cells. J Power Sources 190:271–278
189. Selembo PA, Perez JM, Lloyd WA et al (2009) High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells. Int J Hydrogen Energy 34:5373–5381
190. Selembo PA, Merrill MD, Logan BE (2010) Hydrogen production with nickel powder cathode catalysts in microbial electrolysis cells. Int J Hydrogen Energy 35:428–437
191. Sharma V, Kundu PP (2010) Biocatalysts in microbial fuel cells. Enzyme Microb Technol 47:179–188
192. Sharma Y, Li B (2010) The variation of power generation with organic substrates in single-chamber microbial fuel cells. Bioresour Technol 101:1844–1850
193. Shea C, Clauwaert P, Verstraete W et al (2008) Adapting a denitrifying biocathode for perchlorate. Water Sci Technol 58:1941–1946

194. Sinyak Y (1994) Global climate and energy systems. Sci Total Environ 143:31–51
195. Siu CPB, Chiao M (2008) A microfabricated PDMS microbial fuel cell. J Microelectromech Syst 17(6):1329–1341
196. Sleutels THJA, Hamelers HVM, Rozendal RA et al (2009) Ion transport resistance in microbial electrolysis cells with anion and cation exchange membranes. Int J Hydrogen Energy 34(9):3612–3620
197. Steinbusch KJJ, Hamelers HVM, Buisman CJN (2008) Alcohol production through volatile fatty acids reduction with hydrogen as electron donor by mixed cultures. Water Res 42:4059–4066
198. Steinbusch KJJ, Hamelers HVM, Schaap JD et al (2010) Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. Environ Sci Technol 44:513–517
199. Stirling JL, Bennetto HP, Delaney GM et al (1983) Microbial fuel cells. Biochem Soc Trans 11:451–453
200. Strycharz SM, Woodward TL, Johnson JP et al (2008) Graphite electrodes as a sole electron donor for reductive dechlorination of tetrachloroethene by *Geobacter lovleyi*. Appl Environ Microbiol 74:5943–5947
201. Tandukar M, Huber SJ, Onodera T et al (2009) Biological chromium(VI) reduction in the cathode of a microbial fuel cell. Environ Sci Technol 43:8159–8165
202. Tartakovsky B, Manuel MF, Neburchilov V et al (2008) Biocatalyzed hydrogen production in a continuous flow microbial fuel cell with a gas phase cathode. J Power Sources 182:291–297
203. Tartakovsky B, Manuela MF, Wang H et al (2009) High rate membrane-less microbial electrolysis cell for continuous hydrogen production. Int J Hydrogen Energy 34:672–677
204. Ter Heijne A, Hamelers HVM, de Wilde V et al (2006) A bipolar membrane combined with ferric iron reduction as an efficient cathode system in microbial fuel cells. Environ Sci Technol 40:5200–5205
205. Ter Heijne A, Hamelersa HVM, Sakkes M et al (2008) Performance of non-porous graphite and titanium-based anodes in microbial fuel cells. Electrochim Acta 53(18):5697–5703
206. Thauer RK, Stackebrandt E, Hamilton WA (2007) Energy metabolism phylogenetic diversity of sulphate-reducing bacteria. In: Barton LL, Hamilton WA (eds) Sulphate-reducing bacteria: environmental and engineered systems. Cambridge University Press, Cambridge
207. Thurston CF, Bennetto HP, Delaney GM et al (1985) Glucose metabolism in a microbial fuel cell stoichiometry of product formation in a Thionine-mediated proteus vulgaris fuel cell and its relation to coulombic yields. J Gen Microbiol 131:1393–1404
208. Thrash JC, Van Trump IV, Weber KA et al (2007) Electrochemical stimulation of microbial perchlorate reduction. Environ Sci Technol 41:1740–1746
209. Torres CI, Marcus K, Rittmann BE (2008) Proton transport inside the biofilm limits electrical current generation by anode-respiring bacteria. Biotechnol Bioeng 100:872–881
210. Trinh NT, Park JH, Kim BW (2009) Increased generation of electricity in a microbial fuel cell using *Geobacter sulfurreducens*. Korean J Chem Eng 26(3):748–753
211. Vega CA, Fernandez I (1987) Mediating effect of ferric chelate compounds in microbial fuel-cells with *Lactobacillus plantarum*, *Streptococcus lactis*, and *Erwinia dissolvens*. Bioelectrochem Bioenerg 17:217–222
212. Villano M, Aulenta F, Ciucci C et al (2010) Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. Biores Technol 101:3085–3090
213. Virdis B, Rabaey K, Yuan Z et al (2008) Microbial fuel cells for simultaneous carbon and nitrogen removal. Water Res 42:3013–3024
214. Walker AL, Walker JCW (2006) Biological fuel cell and an application as a reserve power source. J Power Sources 160:123–129
215. Wang AJ, Liu W, Cheng S et al (2009) Source of methane and methods to control its formation in single chamber microbial electrolysis cells. Int J Hydrogen Energy 34: 3653–3658

216. Wang AJ, Liu WZ, Ren NQ et al (2010) Key factors affecting microbial anode potential in a microbial electrolysis cell for H_2 production. Int J Hydrogen Energy 35:13481–13487
217. Watanabe K (2008) Recent developments in microbial fuel cell technologies for sustainable bioenergy. J Biosci Bioeng 106(6):528–536
218. Xing DF, Zuo Y, Cheng S et al (2008) Electricity generation by *Rhodopseudomonas palustris* DX-1. Environ Sci Technol 42(11):4146–4151
219. Xing DF, Cheng S, Regan JM et al (2009) Change in microbial communities in acetate- and glucose-fed microbial fuel cells in the presence of light. Biosens Bioelectron 25:105–111
220. Xi MY, Sun YP (2008) Preliminary study on *E.coli* microbial fuel cell and on-electrode taming of the biocatalyst. Chinese J Process Eng 8(6):1179–1184
221. You SJ, Zhao QL, Zhang JN et al (2006) A microbial fuel cell using permanganate as the cathodic electron acceptor. J Power Sources 162:1409–1415
222. You SJ, Ren NQ, Zhao QL et al (2009) Power generation and electrochemical analysis of biocathode microbial fuel cell using graphite fiber brush as cathode material. Fuel Cells 5:588–596
223. Yuan Y, Kim S (2008) Polypyrrole-coated reticulated vitreous carbon as anode in microbial fuel cell for higher energy output. Bull Korean Chem Soc 29:168–172
224. Zhang J, Zhao Q, Aelterman P et al (2008) Electricity generation in a microbial fuel cell with a microbially catalyzed cathode. Biotechnol Lett 30:1771–1776
225. Zhang T, Cui C, Chen S et al (2006) A novel mediatorless microbial fuel cell based on direct biocatalysis of *Escherichia coli.* Chem Commun 21:2257–2259
226. Zhang YM, Merrill MD, Logan BE (2010) The use and optimization of stainless steel mesh cathodes in microbial electrolysis cells. Int J Hydrogen Energy 35:12020–12028
227. Zhao F, Harnisch F, Schröder U et al (2005) Application of pyrolysed iron(II) phthalocyanine and CoTMPP based oxygen reduction catalysts as cathode materials in microbial fuel cells. Electrochem Commun 7:1405–1410
228. Zhao F, Harnisch F, Schröder U et al (2006) Challenges and constraints of using oxygen cathodes in microbial fuel cells. Environ Sci Technol 40:5193–5199
229. Zhao F, Rahunen N, Varcoe JR et al (2008) Activated carbon cloth as anode for sulfate removal in a microbial fuel cell. Environ Sci Technol 42:4971–4976
230. Zhou MH, Chi ML, Luo JM et al (2011) An overview of electrode materials in microbial fuel cells. J Power Sources 196(10):4427–4435
231. Zhu NW, Chen X, Zhang T et al (2011) Improved performance of membrane free single-chamber air-cathode microbial fuel cells with nitric acid and ethylenediamine surface modified activated carbon fiber felt anodes. Biores Technol 102(1):422–426
232. Zou YJ, Xiang CL, Yang LN et al (2008) A mediatorless microbial fuel cell using polypyrrole coated carbon nanotubes composite as anode material. Int J Hydrogen Energy 33:4856–4862
233. Zou YJ, Pisciotta J, Baskakov LV et al (2010) Nanostructured polypyrrole-coated anode for sun-powered microbial fuel cells. Bioelectrochem 79:50–56

Bioeconomy and Transportation Infrastructure Impacts: A Case Study of Iowa's Renewable Energy

Konstantina Gkritza, Inya Nlenanya and Weiwei Jiang

Abstract The federal government is aggressively promoting biofuels as an answer to global climate change and dependence on imported energy sources. As a result, ethanol production in the United States (U.S.) grew at an annual rate of 32% between 2005 and 2008 spurred on by the U.S. Department of Energy's goal of having 20% of U.S. transportation fuels to come from biological-based sources by 2030. This will require a dramatic increase in the present levels of ethanol and biodiesel production and distribution and have significant infrastructure implications as transportation of raw materials to the biorefineries and biofuel to markets will not only create additional transport demand but also competition and significant growth in other major freight categories. The present chapter first discusses the physical and fiscal impacts of biofuel plants and wind power industries. Then, using a sample of 24 counties in the north, west, and south part of Iowa, which were identified through a local agency survey as having a large number of diverse facilities, one-way panel data regression models are presented that estimate pavement condition and maintenance costs as a function of vehicle miles traveled, plant capacity and years of operation, corn and soybean production, and soil and environmental conditions.

This material is based upon work supported by the Iowa Highway Research Board under project IHRB-07-03. The contents of this chapter reflect the views of the authors, who are responsible for the facts and the accuracy of the data presented herein. The contents do not necessarily reflect the official views or policies of the Iowa Department of Transportation, the Institute for Transportation, or Iowa State University.

K. Gkritza (✉) · I. Nlenanya · W. Jiang
Civil Engineering, Institute for Transportation, Iowa State University, Iowa, USA
e-mail: nadia@iastate.edu

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*, Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_9,

1 Introduction

The United States (U.S.) is the world leader in bioeconomy and renewable energy, having identified the duo as a key pillar in its efforts to reduce dependence on foreign oil (1). In addition, national security concerns, the desire to increase farm incomes, and hosting new and improved technologies have become powerful incentives for the production and use of biofuels (2). The renewable energy industry like any other industry involves production/collection and distribution of raw materials as well as finished products. As a result, the success of the renewable energy industry depends on the quality of service that the transportation infrastructure can provide. Since the U.S. interstate system was completed in the 1980s, investment and innovation in the U.S. transportation infrastructure—roads and bridges, airports, public transit, and railway systems—have lagged behind other leading world economies even though traffic volumes, weight of freight, and legal truck weight limits have all changed considerably (3). An interim report from The National Surface Transportation Infrastructure Financing Commission (NSTIFC) in 2008 confirms that system demands are outpacing investment in the U.S. transportation infrastructure as well as the fact that system maintenance is competing with the necessary expansion of the system (4). This is especially noteworthy considering that the distribution of biofuels is highly transportation-intensive. As a result, for the U.S. to come close to achieving its goal of reaching 36 billion gallon level of biofuels by 2022 (in 2010, biofuel production was nearly 4 billion gallons), there is a need to not only ramp up the investment in infrastructure and technology but also come up with a multimodal policy that will optimize the distribution of biofuels seamlessly across all modes.

Iowa makes a very good case study for the impact of bioeconomy and other sources of renewable energy (such as wind) on the transportation infrastructure. According to the Iowa Department of Economic Development (IDED), Iowa is ranked number one nationally in ethanol and biomass production, second in biodiesel production, and has recently superseded California as the second largest producer of wind energy (5). These milestones come at a price on the state's transportation infrastructure as increased traffic resulting from the renewable energy industry will likely impact the transportation infrastructure condition and increase the maintenance expenses for the state and local governments (6, 7). Currently, 79% of all state, county, and municipal roads in Iowa are under the jurisdiction of a county (8) and most, if not all, biofuel plants or wind farms are located right off a county road or the secondary road system. This results in additional burden of maintenance to the counties which already have more roads under their jurisdiction than they can maintain.

The present chapter first discusses the physical and fiscal impacts of biofuel plants and wind power industries. Then, using a sample of 24 counties in the north, west and south part of Iowa that were identified through a local agency survey as having a large number of diverse facilities, one-way panel data regression models are presented that estimate pavement condition and maintenance costs as a

function of vehicle miles traveled, plant capacity and years of operation, corn and soybean production, and soil and environmental conditions.

2 Impacts Associated with Biofuel Plants

Figure 1 shows the typical transport system involved in the production of biofuels. The truck transportation is associated with ethanol and biodiesel production in four phases; i) farm to storage (sometimes farm to fuel production plant); ii) storage to renewable fuel production plant; iii) fuel production plant to fuel blending and/or storage; and iv) storage to retail markets. For discussion purposes, phases (i) and (ii) can be grouped as moving raw materials to ethanol plants, while phases (iii) and (iv) can be grouped as moving ethanol to market.

2.1 Moving Raw Materials to Ethanol Plants

Under this category of impacts, there are two types of impacts to consider moving corn to the ethanol plants and moving cellulosic materials to ethanol plants.

2.1.1 Moving Corn to Ethanol Plants

The biofuels commonly used in the U.S. include ethanol and biodiesel but much of the discussion is going to be focused on ethanol as it is the main index for tracking biofuel production in the U.S.

In the U.S., ethanol is largely produced in the Midwest. Currently, the primary feedstock for ethanol is corn and most biorefineries are located close to grain production (9). As a result, it makes practical sense to use trucks for short-hauls of corn to the plants. As the volume of production increases, so does the need for intrastate transportation. Specifically, Iowa has the capacity to produce more than 3.9 billion gallons of ethanol annually (more than 30% of the entire U.S. production), about 52% of which is sold to markets outside of Iowa. According to a survey conducted by Iowa State University (10), high demand for ethanol industries is expected to generate more need for intrastate transportation. In addition, building or expanding new and current plants would increase intrastate transportation in the short-term as construction equipment and materials are hauled to the construction site. Typically, the trucks that haul construction materials and equipment are typically heavier than those hauling grain or fuel which means that in the short-term, the impacts due to construction are potentially greater and likely to exacerbate after the plants become operational.

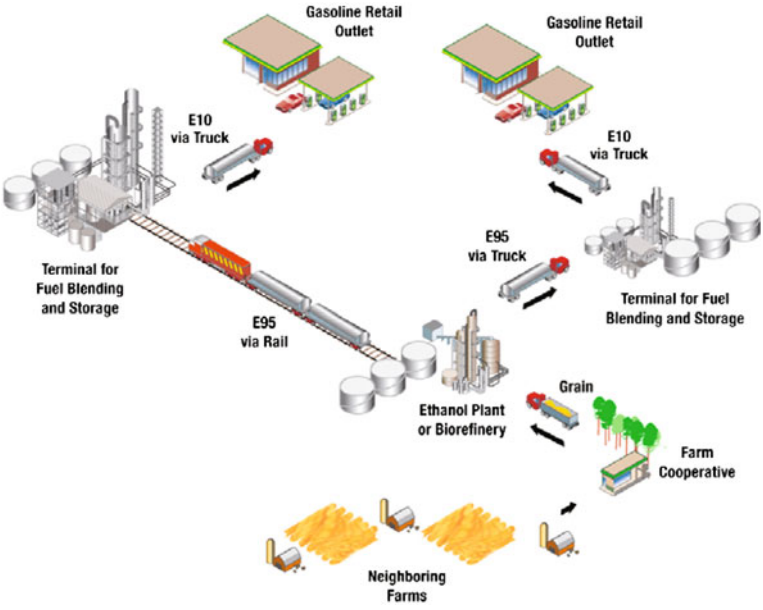

Fig. 1 A typical transport system for ethanol production *(Source: National Bioenergy Center, National Renewable Energy Laboratory)*

2.1.2 Moving Cellulosic Materials to Ethanol Plants

The second impact is related to the cellulosic ethanol plants. Ethanol production from corn alone is capped at 15 billion gallon level by 2015 (11), which falls short of the U.S. Department of Energy (DOE) targets. However, studies have shown that cellulosic materials such as wheat and rice straw, switch grass, paper pulp, and agricultural products (corn cobs and corn stover) can produce more renewable fuel than starches and sugars found in corn (12–15). Consequently, cellulose production for renewable fuels may shift toward genetically-modified perennial grasses such as switch grass and Miscanthus. Cellulosic materials are bulky and will potentially require large-sized vehicles for transportation of materials to processing plants.

Both scenarios present a challenge especially to those sections of the U.S. functionally obsolete infrastructure (such as narrow bridges and roads), which prohibit the movement of large-sized vehicles. The nation's bridges and roads received mediocre and poor grades, respectively by the American Society of Civil Engineers (16). While heavy vehicle movements could be restricted (or limited) on structurally deficient infrastructure (such as load limited bridges, thin pavements, gravel and unpaved roadways), the oversized vehicles are not as easily managed. The challenges to be confronted relate to the weight and the physical dimensions of transportation vehicles used in the renewable fuel production process.

2.2 Moving Ethanol to Market

While ethanol plants are largely located in the Midwest and close to grain production, ethanol is largely consumed on the East Coast, the West coast, and along the Gulf Coast, areas that are characterized by high population densities (17). Ethanol is shipped via truck, rail, or barge to a storage hub, petroleum or blending terminal, and rail-to-truck transloading facilities (truck-to-rail, and truck- or rail-to-barge are intermediate hauls) (18). In 2009, almost 500 petroleum terminals had storage for ethanol, but only 88 of these had access to rail—the mode that transports most of the ethanol produced today (19). Hence in the short-term, while waiting for technology to catch up which will allow the piping of ethanol in the same way as gasoline is moved today, moving ethanol to market will increasingly depend on trucking of ethanol across state lines. In addition, the next generation of ethanol production involving cellulosic feedstock will start out following the same geographic trend as corn production, but then adapt to the transportation infrastructure and policies in place. As a result, while increased ethanol production will ramp up the intrastate transportation, distribution of ethanol will increase significantly the interstate freight, which at current levels is projected to become more than double by 2035. Note that the volume of ethanol being moved would have to triple by 2030 in order to meet the U.S. DOE target.

3 Impacts Associated with Wind Energy Farms

In the U.S., new wind power project installations have been expanding at an average rate of 39% per year for the past five years (20). In 2008, the U.S. DOE examined the feasibility of obtaining 20% of the U.S. electricity from wind energy alone by 2030 (21). According to the American Wind Energy Association (AWEA), ramping up to that level would require the installation of 7,000 turbines a year by 2018 which translates to 50,000 annual shipments of turbine components (22).

The impacts of the wind energy industry on the transportation system are generated during the installation of the wind turbines. The major impact of the wind energy industry on the transportation infrastructure is that of moving turbines and other parts from manufacturing plants to the wind turbine construction sites in addition to moving the machines and equipments that will be used to assemble the turbines on location. A main challenge of moving wind turbines and equipment is navigating a variety of state and local permitting rules for oversized/overweight loads. Moreover, roads and bridges on the secondary system were not designed to accommodate very long and heavy vehicles. As such, moving 50,000 of heavy as well as long configurations would require significant investment in the transportation infrastructure to bring it up to date with the necessary safety countermeasures in place as well as streamlining the weight restrictions in place in various states and locales. However, once installed, wind farms do not generate ongoing heavy vehicle traffic like the biofuel industry does.

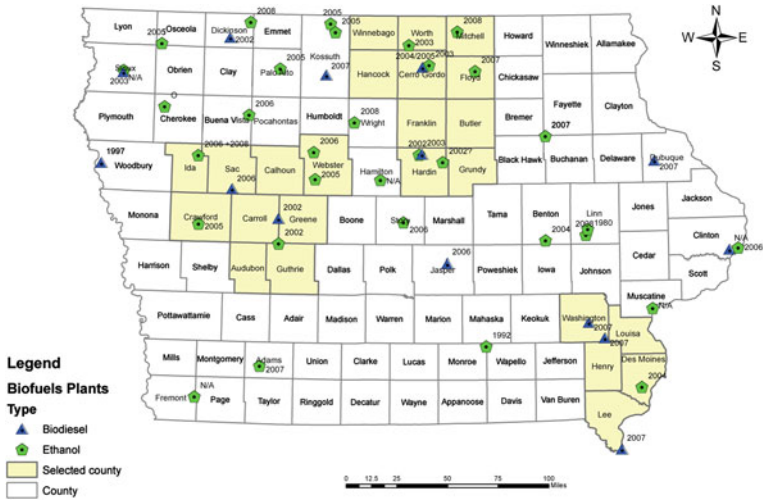

Fig. 2 Selected County Clusters

4 Case Study

4.1 Selected County Clusters

The selection of the counties for study was based on the responses to a local agency survey (96% response rate) and several other considerations. First, selected counties should have at least one operating ethanol or biodiesel plant. Second, it would be preferable to have at least one plant that had been in operation for an extended period of time, so that it would be possible to compare maintenance costs and pavement condition trends before and after operation. Information regarding the plants' operational year was acquired from the survey of county engineers. Third, the selection of a cluster of counties, which include counties surrounding those with biofuel plants, was preferable to the selection of individual counties, since the biofuel plants impacts are likely not constrained by jurisdictional boundaries but can impact adjacent counties. Lastly, the selected clusters should be dispersed across the state to represent a variety of soil, terrain, and environmental conditions. A total of 24 counties in clusters in Northern, Western, and Southern Iowa were selected (shown in Fig. 2).

4.2 Trend Analysis

A trend analysis of historical maintenance cost data and traffic growth was conducted. The data source for the maintenance costs was the annual county expense reports, which are prepared by county engineers and filed annually with the Iowa

Department of Transportation (DOT). These reports utilize a standard accounting system for all road department expenses. The expenses are subdivided into maintenance expense categories that are coded by three digit numbers. A full listing of all potential expense categories is referenced in (23). The cost categories selected for review included only the following:

- 420–bridge repairs (materials and county or contract labor expenses for maintenance)
- 451–blading (road maintenance labor)
- 461–granular (surfacing and hauling labor)
- 466–asphalt pavement repairs
- 467–PC concrete pavement repairs
- 521–snow & ice control (labor for pavements)
- 522–snow plowing (labor for gravel roads)
- along with combinations of all of the above.

These categories were selected as being the most representative where maintenance expenses would increase if there was a fiscal impact on the transportation system due to renewable energy facility constructions or operations.

Traffic volume was used as a measure of traffic load repetitions. Traffic data were based on the annual vehicle miles traveled (VMT) reports that were obtained from the Iowa DOT. Large truck VMT (LVMT) on the secondary road system were of particular interest. Large truck is defined herein as a single or multiple trailer trucks with 4 or more axles.

4.2.1 Maintenance Cost Analysis

Road maintenance costs for the period, 1999 through 2008, were reviewed. Graphs for each of the selected county's data were prepared showing recorded annual expenses by category and indicating the year the facility was constructed. It was expected that the heavy usage of the roads around a biofuel plant or a wind energy farm when it was built would create a visible "spike" or increased cost. These "spikes" could show preliminary evidence that additional costs were associated with the renewable energy facilities. If this were to be true, all of the counties in the cluster would show the same trend or "spike" after the construction of renewable energy facilities.

Overall, it was found that pavement (cost categories 466 and 467) and gravel road (cost categories 451 and 461) repairs were higher in the following year or two years following the beginning of operations of a biofuel plant. This could indicate not only the additional effort required to maintain the road conditions overall, but there may also have been a shift in the level of maintenance provided to a new group of roads (those that were bringing loads to the plant). The review of winter maintenance practices showed several spikes in 2001, 2004, and again in 2008. Upon investigation, historical evidence revealed that Iowa experienced a very severe winter season in 2008. The remaining two spikes could be attributed to the

need for the increased use of roads for delivery of grain to the facilities during the winter season. Lastly, heavy loads associated with the wind farm construction could have caused some spikes on bridge damage repairs. Following the renewable energy facilities openings, bridge damage could also possibly have been caused by extreme stresses due to heavy and repeated loadings of grain carts and trucks. Note that nearly each set of county graphs appeared to have shown some unique spikes and it seemed that there was substantive evidence of increased costs from the renewable energy facilities.

4.2.2 Traffic Growth Analysis

LVMT trends were reviewed over the period 2002 through 2008. VMT data prior to 2002, when most of the wind turbines were installed in the selected counties, were not readily available; the authors looked for any trends around the time the renewable energy facility was under construction and when it became fully operational. The trend analysis findings suggested an increase in truck traffic during plant construction, and while the truck traffic decreased significantly after the plants became operational, it was still higher than the pre plant traffic levels.

4.3 Statistical Analysis

4.3.1 Data

For each of the 24 counties selected for the statistical analysis, information on maintenance-related costs, Pavement Condition Index (PCI), plant capacity and years of operation, traffic volumes, environmental and agricultural factors, and soil condition were collected during the period 1999–2008. Maintenance-related costs and PCI were selected as dependent variables in the estimated model. PCI is a numerical index between 0 (very poor) and 100 (excellent) and is widely used to indicate pavement condition and deterioration over time based on measurements of roughness and surface distress. PCI was collected for both primary and secondary road systems; however, northern counties had not collected PCI data for their secondary road system since 2004. Therefore, only PCI for the primary road system was utilized for this study. In view of these data limitations, maintenance costs as reported in the annual county expense reports, were used as an indirect indicator of pavement condition and deterioration over time. It should be noted that the amount spent on maintenance is based on available funds rather than actual needs and, as such, the magnitude of impacts could be higher than that reflected in the maintenance costs.

Information on annual corn production and soybean production by county was obtained from the U.S. Department of Agriculture (USDA) and the National

Agriculture Statistic Service (24). Environmental factors that affect pavement condition include temperature and precipitation, both of which affect the elastic moduli of the various layers. In this study, snowfall and rainfall depths were considered as proxies for precipitation levels. A freezing index, in terms of degree-days below freezing, also was calculated to capture the effect of temperature on pavement deterioration. Environment-related data were obtained from Iowa Environmental Mesonet (25). The soil condition rating for each county was based on the American Association of State Highway and Transportation Officials (AASHTO) classification system. The selected counties were spatially joined to the U.S. General Soil Map data for Iowa, which was obtained from the USDA (24), and the proportion (percentage) of area in each broad category of soil (poor, fair, and good) per county was estimated. Overall, the pavement subgrades in these counties were rated from poor to fair condition. Table 1 provides the summary statistics of select variables.

4.3.2 Methodology

The dependent variables considered for this study are maintenance cost and PCI. Both are continuous variables, that is, they can take on any value within a range of values (PCI can take on any value between 0 and 100). Therefore, as the dependent variables are continuous, a regression model is developed in order to determine the factors affecting them. As data for different years and counties have been gathered, the data are analyzed as panel data, in order to include the influence of different counties and years on the maintenance cost and PCI.

One-way error model is the simplest and most straightforward model for accounting for cross-sectional heterogeneity in panel data. Because heterogeneity effects are assumed to be constant for the given cross-section units or different cross-sectional units during one time period, they are absorbed by the intercept term as a means to account for individual or time heterogeneity (26). More formally, a panel data regression is written as (27):

$$Y_{it} = \alpha + X'_{it}\beta + \mu_{it}, \quad i = 1, \ldots, n; \quad t = 1, \ldots T \tag{1.1}$$

where i refers to cross-section unit (counties), and t refers to time; α is constant, β denotes coefficients of explanatory variables, X'_{it} denotes a set of explanatory variables, independent of vit for all i,t. Disturbances of one-way error components model are given as:

$$\mu_{it=}\mu_i + v_{it} \quad i = 1, \ldots, n; \quad t = 1, \ldots, T \tag{1.2}$$

where μ_{it} denotes the unobserved cross-sectional specific effect, v_{it} denotes random disturbances, μ_i are assumed to be fixed parameters to be estimated and the v_{it} are random disturbances that follow the usual regression assumptions (28).

In addition, two modeling specifications are considered: fixed- and random-effects. The essential difference between these two modeling specifications is

Table 1 Summary statistics

Variable description	Mean value	Standard deviation	Cases
Dependent Variables			
Pavement cost (dollars)	97196.3	106470	225
Granular and blading cost (dollars)	869265	340556	231
Winter maintenance cost (dollars)	101767	79077.7	231
Bridge maintenance cost (dollars)	41202.3	36616.2	231
PCI	66.237	7.585	240
Independent Variables			
Primary rural VMT	1.62E + 08	1.53E + 08	240
Primary urban VMT	7.42E + 07	1.47E + 08	240
Primary rural LVMT	2.89E + 07	3.57E + 07	240
Primary urban LVMT	7.72E + 06	1.73E + 07	240
Secondary LVMT	2654.41	1067.15	168
Secondary VMT	48490.2	19420.6	216
Local LVMT	333.464	494.51	168
Local VMT	31318.5	37644.2	216
Corn production (bushel)	2.24E + 07	7.30E + 06	240
Soybean production (bushel)	5.18E + 06	1.53E + 06	240
Snow depth (inch)	306.11	231.3	240
Rainfall depth (inch)	34.37	7.0	240
Freezing index (degree-days)	2263.3	484.23	230
Ethanol plant present (1 if present, 0 otherwise)	0.179	0.38	240
Biodiesel plant present (1 if present, 0 otherwise)	0.1	0.30	240
Wind farm present (1 if present, 0 otherwise)	0.16	0.37	240
Number of ethanol plants	0.22	0.50	240
Number of biodiesel plants	0.11	0.34	240
Number of wind turbines	8.64	26.32	240
Years of operation of ethanol plant	0.59	1.46	240
Years of operation of biodiesel plant	0.33	1.14	240
Years of operation of wind farm	0.55	1.60	240
Capacity of ethanol plant (million gallons)	15.05	35.81	240
Capacity of biodiesel plant (million gallons)	2.6	9.00	240
Soil condition–good (%)	4.33	8.18	240
Soil condition–fair (%)	40.96	24.39	240
Soil condition–poor (%)	54.79	23.58	240
Freezing index in northern counties (degree-days)	1012.72	1231.97	240
Soil condition–fair in southern counties (%)	9.75	19.58	240
Soil condition–fair in northern counties (%)	16.54	25.05	240
Soil condition–poor in southern counties (%)	10.13	20.90	240
PCI in southern counties	14.27	28.06	240

whether the inference from the estimated model is confined to the effects in the model, or the inferences are made about a population of effects. In the former case the fixed-effects model is appropriate, while the latter case is suited for the random-effects model (27, 28). In this study, the estimation results of a

fixed-effects model can explain the maintenance costs and pavement conditions in the 24 selected counties, whereas the estimation results of a random-effects model apply to the whole state.

4.3.3 Estimation Results

One-way fixed-effects models were estimated to determine the effects of the independent variables on maintenance costs and pavement condition in the 24 selected counties, while one-way random-effects models were applied to extend the analysis to the entire state. The parameter estimates and corresponding *t*-statistics are presented in Table 2. All variables were significant at the 90% level of confidence or higher.

When considering the overall performance of the models, the fixed-effects model was found to have a higher *R*-squared value, which means that the fixed-effects model explained well the factors that affect pavement condition and maintenance costs in the 24 counties. However, the random-effects model was able to explain more variables and extrapolate the results to the entire state, albeit at a lower level of confidence. For this reason, both model results are included and discussed in the present chapter.

The panel data regression models explained satisfactorily (as indicated by the high *R*-squared values) the granular and blading costs, winter maintenance costs, and pavement condition index. However, the model did not explain bridge maintenance costs very well. This might be because the factors (such as structural design, construction detail, and material type) that affect bridge condition were not included in the current model. Note that the county engineer interviews revealed that most county engineers have not noticed damage on their bridges that they could relate directly to the biofuel plants or wind energy farms. However, they agreed that the hauling of raw material to the plants would continue to cause distress to their aging bridges.

Overall, during the study period (1999–2008) the pavement condition of primary roads deteriorated, while the maintenance cost for granular and blading and winter maintenance increased. Regarding traffic volume factors, LVMT, especially local LVMT, was found to have a better correlation with road and bridge maintenance costs. This confirms that large truck traffic is a significant factor affecting infrastructure condition and deterioration.

Biofuel plants and wind farms were both found to affect road pavement condition and maintenance costs, but their effect showed different patterns. The pavement condition index model indicated that the higher the capacity of biodiesel or ethanol plants in a county, the worse the road pavement condition was. This shows that after the biofuel plants began operation, road pavement condition more likely deteriorates. For example, the operation of a 100-million gallon ethanol plant would result in a reduction of PCI by 2 points, while a similar effect is expected due to the operation of a 30-million gallon biodiesel plant. Turning to maintenance costs, it was estimated that adding one-million gallon ethanol plant

Table 2 Panel data regression model estimation results

Variable	Fixed-effect model		Random-effect model	
	Coefficient	t-statistic	Coefficient	t-statistic
Dependent Variable: Pavement Maintenance Costs				
Constant	–	–	70,966	2.23
Year of 2006	26,264	1.49	–	–
Year of 2005	–	–	−3,3657.9	−1.93
Soybean production	−0.02	−2.73	–	–
Rainfall depth	2,441.63	2.95	1,999.0	2.40
Capacity of ethanol plant	589.50	2.86	701.02	3.78
Soil condition-good	–	–	−3,180.4	−2.44
Soil condition-fair in northern counties	–	–	−1,038.31	−2.26
PCI in southern counties	–	–	−1,348.08	−3.37
R-Squared	0.523		0.276	
Dependent Variable: Granular and Blading Maintenance Costs				
Constant	–	–	768,571	13.20
Year	11,825	1.49	–	–
Year of 2006	–	–	53,547	1.49
Local LVMT	88.90	1.66	128.36	2.99
Soybean production	0.03	1.56	–	–
Number of wind farms	−1,154.14	−1.59	−967.02	−1.44
Capacity of biodiesel plant	4,275.20	2.21	5,007.23	2.71
Soil condition-fair in southern counties	–	–	6,254.00	2.41
R-Squared	0.819		0.275	
Dependent Variable: Winter Maintenance Costs				
Constant	–	–	−94,792.82	−3.38
Year	4,348	3.13	3,172.41	1.87
Primary rural LVMT	–	–	0.0005	2.36
Secondary VMT	–	–	1.49	4.23
Capacity of biodiesel plant	–	–	−1,000.13	−2.03
Snow depth	167.22	9.93	165.56	9.51
Rainfall depth	969.60	1.58	1,287.67	1.91
Freezing index in northern counties	–	–	11.22	1.92
Soil condition-good	–	–	−2,052.20	−2.27
R-Squared	0.638		0.458	
Dependent Variable: Bridge Maintenance Costs				
Constant	–	–	−4674	−0.31
Local LVMT	11.64	1.32	14.41	2.33
Soybean production	0.004	1.03	0.01	2.43
Wind farm present	–	–	−20,200	−2.57
Soil condition-poor	–	–	343.27	2.25
R-Squared	0.360		0.100	
Dependent Variable: PCI				
Constant	–	–	59.60	28.03
Year	−0.21	−1.80	−0.19	−1.54
Primary rural LVMT	−0.39D−06	−3.37	−0.44D−07	−1.61

(continued)

Table 2 (continued)

Variable	Fixed-effect model		Random-effect model	
	Coefficient	t-statistic	Coefficient	t-statistic
Soybean production	0.71D−06	1.76	–	–
Snow depth	0.003	1.86	–	–
Rainfall depth	–	–	0.08	1.79
Capacity of ethanol plant	−0.02	−1.50	−0.02	−1.34
Capacity of biodiesel plant	−0.05	−1.27	–	–
Freezing index in northern counties	–	–	0.002	3.36
Soil condition-poor in southern counties	–	–	0.13	2.51
R-Squared	0.717		0.267	

would increase pavement maintenance costs by $590, while adding one-million gallon biodiesel plant would increase granular and blading maintenance costs by $4,275. For example, the operation of a 100-million gallon ethanol plant would result in an increase in pavement maintenance costs by $59,000, while the operation of a 30-million gallon biodiesel plant is expected to increase granular and blading maintenance costs by $128,250.

Contrary to this, it was found that the higher the number of wind turbines in a county, the lower the maintenance costs were. This indicates that county road maintenance costs were lower after wind farms became operational. In other words, wind farms may cause damage to the roads during the construction period, but once the wind turbines are installed, there might be no further road deterioration. Unlike ethanol and biodiesel plants that require regular transportation of raw material and final products during operation, wind farms impact the road system only during the construction period. However, according to the county interviews, the maintenance expenses related to a wind farm might not be fully captured. For example, in Mitchell County, the construction company did all the crushing and hauling of the aggregate necessary to maintain and repair the granular roads. Therefore, no cost records were available for this expense. Floyd County also experienced very little road damage because most of their wind turbines were installed during the winter months when the roads were frozen.

The agricultural factors showed little or no correlation with maintenance costs and pavement condition. The variable for corn production was not found to be significant, and although soybean production was found to be significant in the maintenance cost models, the effect was very low. This could be likely attributed to the high correlation of these variables with the capacity of ethanol and biodiesel plants. However, the effect of soybean production was found to be significant in the pavement condition model. Higher soybean production may lead to better pavement condition. This could be because soybeans weigh less than corn, and corn–soybean rotation is common in farming practices. Therefore, more soybean production could translate to less corn production, and overall lightweight agricultural product to be transported.

Environmental factors, including snow depth, rainfall depth, and freezing index, were found to have a high and positive correlation with pavement and/or winter maintenance costs, and interestingly with PCI. It is possible that counties spend more money on repairing the road after a harsh winter (which in turn was reflected in higher winter maintenance costs), and so the road pavement condition improved after the maintenance activity had been performed. The soil condition also had a high correlation with maintenance costs. The higher the percentage of good soil in a county, the lower the maintenance costs were and vice versa.

5 Discussion

The U.S. continues to maintain its position as a leader in the bioeconomy and wind energy production, but meeting the U.S. DOE's goal of having 20% of the transportation fuels in the U.S. that come from biological-based sources by 2030 will require a dramatic increase in ethanol and biodiesel production and distribution. Increasing ethanol and biodiesel production will place stress on the infrastructure involved in producing and shipping raw material and refined products. Trucks are used as the main transportation mode to move corn and soybean from farm to biofuel processing plants, and semi-trucks are the most preferable mode within truck transportation. With the ongoing semi-truck traffic surrounding biofuel processing plants, deterioration of roads' condition can be expected. As wind turbine blades are getting longer and heavier, transportation from manufacturing sites to the wind farms also is becoming problematic.

This chapter investigated the physical and fiscal impacts of existing biofuel plants and wind power industries, using Iowa as a case study. The maintenance-related expense analysis showed a trend of increased maintenance costs in the year after a biofuel plant became operational, as well as during the construction period. The traffic growth analysis showed that large truck traffic increased dramatically during the construction period, then dropped after the plant became operational, but not to the levels prior to the construction of the plant.

Furthermore, one-way-error panel data regression models were developed to estimate pavement condition and maintenance costs as a function of vehicle miles traveled, plant capacity, and years of operation, corn, and soybean production, soil condition and environmental factors. Twenty-four counties in the north, west and south part of the state were selected for analysis. The estimation results indicated that large truck traffic contributed to road deterioration at a higher degree compared to other highway modes of transportation, as expected. Road damage from traffic loads were associated with both the biofuel industry and wind farms, but in different patterns. For wind farms, the major damage occurred during construction activities and predominantly on gravel roads. These roads impacted were used as haul roads for heavy transformers, other turbine parts, and multiple loads of construction materials. For biofuel plants, the road damage that occurred was not only during plant construction, but the damage was on going due to the continual

hauling of raw products to the plant and finished products to the market. The larger the capacity of the biofuel plant, the greater the pavement infrastructure deterioration and associated maintenance costs. Besides traffic loads and biofuel plant capacity, environmental factors and soil condition were also highly significant in explaining maintenance costs and pavement condition

While this analysis provided valuable insights on the renewable energy industry and associated infrastructure impacts, some limitations of this study should be noted. First, pavement condition index data, which is the most direct measure of road condition, was mostly available for primary roads. As such, it was not possible to directly measure the impact of the renewable energy-associated traffic on the condition of the secondary and local roads. Instead, maintenance costs were used as an indirect indicator of pavement condition and deterioration over time. However, maintenance costs do not fully represent road damage in need for repair but only the spending budget allocated. Therefore, if the maintenance costs were determined fully by "needs for repair" and not the allocated budget, the results might have shown higher county maintenance costs and more severe impacts of the biofuel plants and wind energy farms on Iowa's road infrastructure.

References

1. Wakeley HL, Hendrickson CT, Griffin WM, Matthews HC (2009) Economic and environmental transportation effects of large-scale ethanol production and distribution in the United States. Environ Sci Technol 43(7):2228–2233
2. Worldwatch Institute (2006) Biofuels for transportation, global potential and implications for sustainable agriculture and energy in the 21st century a national leader in clean energy, http://www.worldwatch.org/system/files/EBF038.pdf Retrieved December 14, 2010
3. Urban Land Institute and Ernst and Young (2007) Infrastructure 2007: a global perspective. Urban Land Institute, Washington, p 19
4. National surface transportation infrastructure financing commission (2008) The path forward funding and financing our surface transportation system, interim report of the national surface transportation infrastructure financing commission, http://financecommission. dot.gov/Documents/Interim%20Report%20-%20The%20Path%20Forward.pdf, Retrieved December 14, 2010
5. Iowa department of economic development (2009) A national leader in clean energy, Iowa life changing, 21(2)
6. Haddad MA, Anderson PF, Thol S, Hertel C, Schmidt B (2009) Assessing the future of the bioeconomy in Greene county Iowa. J Rural Community Dev 4(1):15–33
7. Fox WF, Porca S (2001) Investing in rural infrastructure. Int Reg Sci Rev 24(1):103–133
8. Boeckenstedt R, Gkritza K, Souleyrette R, Nambisan S (2010) Implications of bioeconomy-based fuel production to the transportation infrastructure in Iowa. Transportation Research Record. J Transp Res Board 2191:84–89
9. Jacobs J (2006) Ethanol from sugar—what are the prospects for US sugar c-ops? rural cooperatives 73(5)
10. Yu TH, and Hart CE (2008) The 2006/07 Iowa grain and biofuel flow study: a survey report, food and agricultural policy research institute (FAPRI) Publications 08-sr102, Food and agricultural policy research institute (FAPRI). Iowa State University, Iowa
11. United States department of agriculture (2010) Transportation in study of rural transportation issues, p 139

12. University of Illinois (2006). Agronomic field trials—research plots, http://miscanthus.illinois.edu/?page_id=14
13. University of Illinois (2006). Giant miscanthus growers guide, 2006. p 3 http://www.miscanthus.uiuc.edu/wp-content/uploads/growersguide.pdf
14. University of Illinois (2006). Biomass energy. http://miscanthus.illinois.edu/?page_id = 21
15. Carroll A, Somerville C (2009) Cellulosic biofuels. Annu Rev Plant Biol 60:165–182
16. American society of civil engineers (2005) Report card for America's infrastructure http://www.infrastructurereportcard.org/sites/default/files/RC2009_transportationl.pdf Retrieved January 10, 2010
17. United States department of agriculture (2010) Transportation in study of rural transportation issues, p 133
18. United States department of agriculture (2010) Transportation in study of rural transportation issues, p 135
19. United States department of agriculture (2010) Transportation in study of rural transportation issues, p 136
20. American wind energy association (2010) Winds of change—a manufacturing blueprint for the wind industry http://www.awea.org/documents/reports/BGA_Report_062510_FINAL.pdf Retrieved December 16, 2010
21. United States department of energy (2008) 20% Wind energy by 2030—increasing wind energy's contribution to US electricity supply http://www1.eere.energy.gov/windandhydro/pdfs/41869.pdf Retrieved December 16, 2010
22. American wind energy association (2008) Wind industry transportation opportunities & challenges http://www.awea.org/documents/factsheets/transportation.pdf Retrieved December 16, 2010
23. Iowa department of transportation (2005) Instructional memorandums to county engineers, July 2005. http://www.iowadot.gov/local_systems/publications/county_im/im_2_071.pdf
24. United States department of agriculture. National agriculture statistic service http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/
25. Iowa environmental mesonet, Iowa State University department of agronomy http://mesonet.agron.iastate.edu/GIS/
26. Hsiao C (2003) Analysis of Panel Data, 2nd edn. Cambridge University Press, UK
27. Baltagi B (2008) Econometric analysis of panel data, 4th edn. John Wiley, UK
28. Washington SP, Karlaftis MG, Mannering FL (2011) Statistical and econometric methods for transportation data analysis, 2nd edn. Chapman&Hall/CRC, Florida

Assessing the Environmental Risks and Opportunities of Bioenergy Cropping

Les Firbank

Abstract All forms of cropping influence the environment, and bioenergy cropping is no exception. The main potential environmental benefit is the net reduction in greenhouse gas (GHG) emissions by the substitution of fossil fuels, while the main potential harm is increased pressure on land use, which can lead to competition for food production, loss of forests and the release of large amounts of carbon from soils and vegetation. The major approaches to environmental risk evaluation are experiments, environmental risk assessment, life cycle analysis, ecosystem services and post-market monitoring; while none are ideal, all these have a potential role in evaluating bioenergy cropping. Major environmental impacts vary greatly between crops, countries and management regimes. Bioenergy cropping has the most positive environmental impact when the crops are productive, have low water and nutrient requirements and can be grown on low-grade and abandoned agricultural land in arrangements that promote biodiversity. Such cropping may be able to supply around 8% of the global energy demand: bioenergy cropping should be seen as one element in a wider strategy for efficient use of land, energy, food and water.

1 Introduction

All forms of land use have environmental impacts. Bioenergy cropping, like any form of agriculture and forestry, impacts upon water quality and availability, on biodiversity and on landscape character. Unlike many other forms of land use,

L. Firbank (✉)
Institute of Integrative and Comparative Biology, University of Leeds, Leeds, LS2 9JT, UK
e-mail: l.firbank@leeds.ac.uk

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*, Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_10,

bioenergy cropping has the potential to substitute fossil fuels, reducing the net emissions of carbon to the atmosphere, thereby potentially reducing climate change. This is because the carbon released from bioenergy crops has only recently been captured from the atmosphere. However, this benefit cannot be fully realised, because bioenergy cropping (the planting, management and harvesting of plants to produce energy) generates greenhouse gases (GHGs) by disturbing the soil and any pre-existing vegetation, and by using the fossil fuels for transport, machinery operation and the development of any inorganic fertilisers and chemical inputs. Other GHG emissions arise from transport and processing between the field and the point at which the energy is used. The goal of this chapter is to look at all of these environmental implications, and to examine how they can be addressed as part of the design and conduct of bioenergy production systems. The focus is on cropping, rather than on other forms of bioenergy production, such as the use of algae and the use of food and agricultural wastes for anaerobic digestion, and rather than on distribution of goods and environmental impacts arising from processing and usage of bioenergy crops.

1.1 The Drive to Increase Global Bioenergy Production

There is nothing new about using plant materials to provide energy; wood has been an important fuel since man first started to use fire. In 1971, 29% of energy used in southern and eastern Asia was from wood, and 66% in Africa (UN statistical yearbook 1973, quoted in [1]). Energy is now derived from a very wide variety of biological materials, including crops, agricultural waste materials and wood (Table 1). While the total supply of bioenergy has increased over recent decades, this increase has been dwarfed by that of coal, gas and oil (Fig. 1).

Concerns about energy security and greenhouse gas emissions have resulted in policies seeking to replace some of these fossil fuels by bioenergy crops. Such policies were pioneered in Brazil, and adopted by the US, the EU and others during the 2000s. During the mid-1970s, global bioethanol production was around 551 million L, rising to 16,940 million L in 2000, 65,690 million L in 2008, and biodiesel production had risen to 14,574 million L from 11 million L in 1991 [3]. Much greater increases are expected. These policies represent a major change in global land use, especially when demands for food production are expected to increase dramatically [4]. To date, production of liquid fuels from biological materials (biofuels) has almost entirely used food crops as feedstocks [3]. These so-called first generation biofuels include maize, sugar cane, oily seed crops and sugar beet. Much greater use is planned for second generation biofuels, notably trees (e.g. willow and poplar), grasses (*Miscanthus*) and oily plants (e.g. *Jathropa*) [3]; such crops are expected to outproduce first generation crops in the US by 2022 [3]. It is possible to use grass for bioenergy instead of as feed for livestock [5–7]. Looking further ahead, algae is being developed as a feedstock, requiring management systems quite unlike current agriculture and forestry [8].

Table 1 Classification of forms of biomass that can be used to generate energy. From [2]

Virgin wood			Fuel wood, forestry residues, tree surgery residues
Energy crops			Agricultural energy crops, grasses and non-woody energy, short rotation coppice, short rotation forestry, aquatics
Agriculture	*Dry residues*		Maize stoves, straw and husks, animal litter
	Wet residues		Grass silage, animal slurry and manure
Waste	*Food waste*		Wet food waste, food oils
	Industrial waste and co-products	Woody waste	Untreated wood, treated wood, wood composites and laminates
		Non-woody waste	Paper pulp and waste, textiles
	Domestic waste		Household food waste, sewage sludge

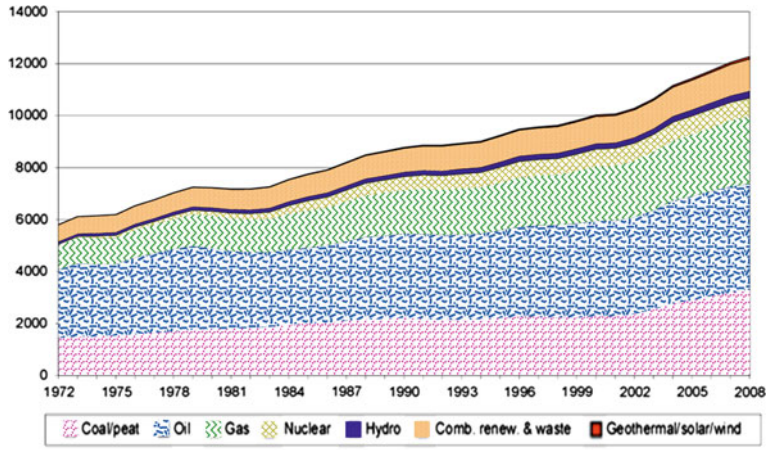

Fig. 1 Global increase in total energy supply by source, million of tonnes of oil equivalent. From International Energy Agency [9]. © OECD/IEA 2010

The environmental footprint of bioenergy cropping will therefore increase substantially, along with the potential benefits and disbenefits. The better they are understood, the more likely it is that the benefits can be maximised.

1.2 The Assessment of Environmental Impacts

Several techniques have been developed that have proved valuable for evaluating environmental risks and benefits for changes in land use and management.

1.2.1 Experimental Comparisons

One approach to assessing the environmental risks and benefits of growing crops (whether for biofuels or for other purposes) is to actually grow them and look for undesirable environmental outcomes. Such experiments can be undertaken in the lab or the field. The design of any such experiment forces one to think clearly about what kind of effect is being tested for, the magnitude of effect considered to be important, what are the appropriate environmental indicators, what are the appropriate experimental conditions and range of receiving environments, how long the experiments should be conducted for.

As all living organisms affect their environment, environmental risks and benefits of growing a particular crop only have meaning when compared with another system. The choice of the comparison is therefore critical. In the case of the replacement of a food crop by a biofuel crop of the same species, it makes sense to compare the two. An appropriate null hypothesis is that there is no difference in terms of environmental impact between one type of crop and the other.

However, one can never prove a null hypothesis in science, only disprove it, and, indeed, much of the scientific debate around environmental risk assessments of GM crops (an analogous situation) has focussed on whether or not the experiments were large enough, and lasted long enough, to detect impacts, expected and unanticipated. In the UK in 1999, concern was being expressed that the introduction of herbicide-tolerant genetically modified crops would have a negative environmental impact, as the broad-spectrum herbicides used with the crops would reduce the number of plants, invertebrates and ultimately birds and mammals in and around the fields [10]. This environmental risk was assessed using the UK Farm Scale Evaluations, that compared GM and non-GM varieties of the same crop. As the major environmental risk identified in advance was the effects of the herbicide regimes associated with the different kinds of crops, rather than from the crops themselves, the treatments required that both GM and non-Gm crops were subject to the management regimes commercially appropriate to each [11]. Plant and invertebrate populations were studied in great detail. The experiment was designed to have an 80% chance of detecting 1.5-fold differences in the selected biodiversity indicators, on the basis of pre-existing knowledge of the variation of these indicators between fields [12]. This specification may appear undemanding, but actually required around 60 replicates per crop, with half-fields used as plots, making it the largest agro-ecology experiment undertaken.

However, the choice of the most appropriate comparator is less obvious for 2nd generation crops. Should it be the previous land use? Perhaps a pre-existing analogue to the proposed bioenergy crop, e.g. a woodland could act as a comparator for short rotation coppice (SRC), but even then, should one use a semi-natural woodland, or a new plantation? If the risk concerns biodiversity, then comparisons become even harder, as different species will inhabit crops of differing structure anyway [13]. Thus, Haughton et al. [14] looked at butterflies at bioenergy crop edges, comparing the results to pre-existing, large-scale data from

field edges around arable fields, reporting that both short rotation coppice (SRC) willow and *Miscanthus* had greater number of butterflies than arable fields. However, the authors stress that whether such comparisons are meaningful is, of course, another point entirely.

This approach is most suitable to investigate a particular kind of environmental risk in detail, rather than to be applied to every possible case [15]. This is because it is both restricted in scope and too expensive to be carried out as a matter of routine [16, 17].

1.2.2 Environmental Risk Assessment

A simple way to evaluate the environmental impacts of land use change is to ask whether there is any environmental risk and, if so, how serious it is and whether it can be avoided. Instead of trying to value all the positive and negative impacts, this approach simply asks, is the new type of crop likely to cause more environmental harm than what it might replace? Again, the environmental risk assessments associated with the use of genetically modified cropping in Europe provides an interesting model. The cultivation of GM crops is subject to EU regulation, one of the conditions being that the environmental risks are no worse than for comparable conventional crops. The burden of proof lies with the organisation wishing to introduce the GM crop.

The risk assessment should follow a logical framework and requires the clear formulation of the problem [18] (Fig. 2). This entails identifying potential environmental hazards and their severity, along with the probability that the hazard will actually be realised. Such potential hazards must be characterised; they may include, for example, changes in land use, cultivation practices and the use of pesticides. These are only environmental hazards if they impact on environmental parameters considered to be important; they might include the presence of rare species, risk of pollution incidents, net releases of GHGs. The selected parameters act as 'assessment endpoints'. They must be measurable, using some form of indicator. The risk assessment involves relating each 'measurement endpoint' to the environmental hazard, experimentally or using pre-existing data or models. For some hazards that can only be detected at large spatial scales, for example impacts on wild populations, only qualitative [19] or quantitative models [20] are available. For others, notably toxicology, there is value in using laboratory studies to test for a relationship between the hazard and the assessment endpoint. In all cases, the more clearly the problem is formulated, the easier it is to define an appropriate experiment to test it [18, 21]. In some cases, the study might conclude that the environmental risk is real, but can be managed. For example, the UK Farm Scale Evaluations showed that the improved weed control in GM herbicide-tolerant beet crops reduced invertebrate and plant food resources higher up the food chain [19]. However, this problem could be mitigated by leaving less than 5% of a field of GM beet unsprayed by herbicides [22].

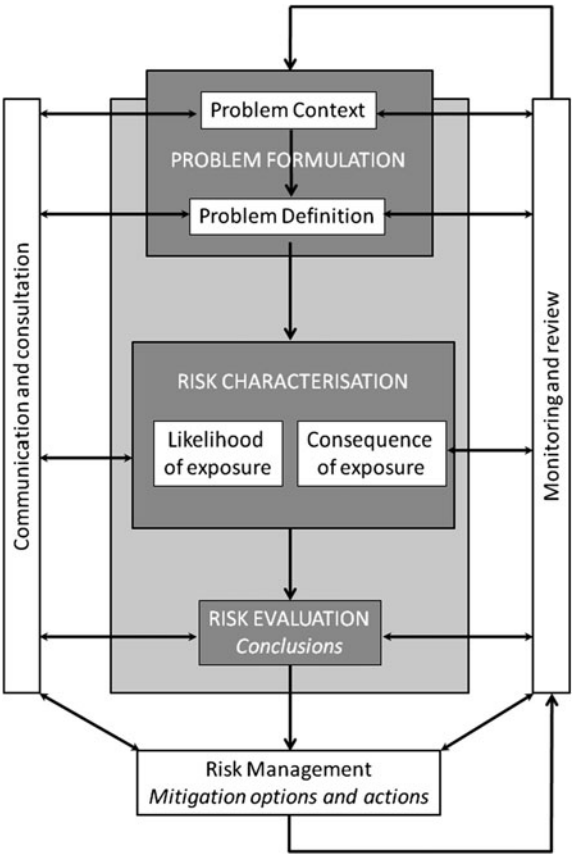

Fig. 2 Problem formulation for environmental risk assessment. The problem context develops the parameters and identifies constraints for the risk assessment, which need to take into legal and regulatory issues. The problem definition ensures that the analysis is manageable and not open ended; it is always case-specific, and depends on the crop being assessed, how it is managed and the receiving environment. It is then possible to characterise the risk of the problem being realised, which is the basis for the evaluation, subject to possible management or mitigation of the risk. A review process is required to ensure that the risk assessments are kept up to date. Communication and transparency are essential throughout the process. Redrawn from [18]

Other approaches may be needed to identify the probability that the hazard may be realised. For example, in response to, concern that the introduction of GM oil seed rape *Brassica napus* into the UK might lead to gene flow to wild relatives *B. rapa* and *B. oleracea*, Wilkinson and colleagues [23] sought to quantify gene flow between current commercial varieties of oil seed rape and wild relatives. They first sought to identify those situations where the two species were co-located, using ground surveys and remote sensing, visited each site to look for wild relatives, testing each to see if they were hybrids between wild and cultivated species. They found that co-location between crops and wild relatives were rare, and only one hybrid plant was actually found in this very large scale study.

Another example concerns the use of GM plants modified to have insecticidal properties. It was suggested that the charismatic Monarch butterfly (*Danaus plexippus*) may be at risk from the commercial use of Bt maize in the US, as the larvae may ingest maize pollen which fell on their host plants, found in maize fields. In practice, the expression of the toxin in pollen was low in most varieties

then available; levels of toxin likely to be encountered in the field did not have acute effects on larvae; there was limited overlap between pollen shed and the larval feeding period; most monarch larvae do not live in or near corn fields, and at the time of the study, only 20% of the maize crop had this trait. Taken together, these factors indicated that the overall risk to monarch numbers from the cultivation of GM maize was negligible at the time [24].

It is very hard to take benefits into account with this approach. In terms of bioenergy cropping, it would be most undesirable should a proven but minor environmental risk outweigh a proven but major environmental benefit, for example, a slight risk of the crop plants becoming locally invasive should not outweigh potential major savings of GHG emissions [13].

1.2.3 Life Cycle Assessment

A common way of addressing environmental impact is to use life cycle assessment (LCA). This is because it is designed to look at all parts of the production system "from cradle to grave"; it can readily address positive and negative impacts, and also because procedures are in place about how they should be conducted [25]. For bioenergy projects, it is often used to address energy and GHG emissions, but can also be used to analyse other factors.

The idea is to quantify carbon sequestration and GHG emissions across the whole system of production per stated level of output (the 'reference flow'), for example 1 MJ of combustable energy. Clearly, photosynthesis and respiration by the crop plants are important, as are the fluxes of nitrous oxide and methane from the soil while the plants are growing. The fluxes associated with land cultivation, crop management, harvesting, transport and processing are clearly relevant. The system can readily be broadened, to include the production and transport of fertilisers and pesticides, indirect effects on land use, the construction of the processing plants, and so on (Fig. 3). The outcome of the analysis therefore depends on its scope (the system boundary), and the choice of components within it (together these comprise the system inventory). Wider system definitions are more inclusive, but can become compounded with other systems, notably food production.

It is important to quantify each component of the system (these comprise the system inventory) and the transfers between them. In some cases, this is straight forward: for example, crop yield and fertiliser application data are normally available from the farmer. In other cases, for example the emissions of GHGs from the soil following fertiliser applications, direct data are rarely available and so pre-existing models can be used [27]. While there are good reasons why individual LCAs are constructed differently, these differences result in substantial variation in the results [26]. Indeed, such variation is potentially so large that some authors doubt whether LCA is an appropriate tool for generalised evaluations of the environmental footprint of bioenergy crops [28].

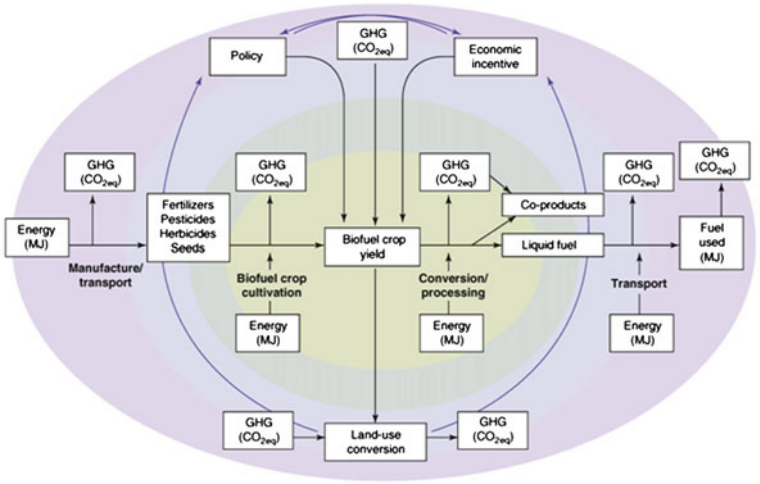

Fig. 3 A chain of production for biofuels with energy and GHG requirements (inputs) and emissions (outputs) defined at each step in the production process, showing the importance of defining the system. The smallest possible system boundary in this case would include only 'Biofuel crop yield', where inputs of GHG would include the CO2 required for photosynthesis and outputs of GHG would include CO2 from autotrophic and heterotrophic respiration, as well as NOx and CH4 fluxes from the soil. Different colours give boundaries to more inclusive systems. Figure from [26]

LCAs that compare different crops and management systems using a common methodology are therefore particularly valuable. One such was undertaken by Rettenmaier et al. [29] for a major review of European bioenergy crops, including carbon balance, acidification, eutrophication, atmospheric quality and potential impacts on human health, each addressed in terms of credits and expenditure for each stage of production from 'cradle to farm gate'. The units of these were standardised by dividing absolute values against reference information, in this case, average energy demand and emissions into the environment per capita in the EU, giving what is termed 'inhabitant equivalents'. Land use change was included in terms of conversion from fallow and cultivated land, with scenarios of indirect effects of land use in Brazil. Biodiversity and landscape quality were not addressed. It turned out that the final analysis of which bioenergy crops are most environmentally beneficial is sensitive to where the crops are sown across Europe, how they are managed and also to how the different environmental categories are weighted with respect to each other; thus, herbaceous crops such as *Miscanthus* deliver greater reductions in GHG emissions than woody crops, but result in levels of acidification and eutrophication (Table 2).

1.2.4 Ecosystem Services

The idea behind ecosystem services is that the earth's environment is not an optional extra in life, but provides benefits that are essential to our well being and that are of value to people. For example, drinking water and food would be unavailable without the provision of clean water and food from the environment, which in turn depend on ecological processes such as nutrient cycling, pollination, plant growth and soil formation [30]. These services can be broken down into different types. Provisioning services are those that generate products for human consumption, namely food, fresh water, fibre, energy etc. Cultural services are those non-material benefits, including sense of place and beauty. These services are underpinned by regulating services, influencing water flows and climate for example, and supporting services such as soil formation, nutrient cycling and primary production (Table 3) [31]. Ecosystem services are all provided by plants, animals and micro-organisms in both managed and natural habitats. Therefore, the environmental degradation observed across much of the earth affects people directly, by reducing the capacity to produce the ecosystem services we need at a time of increasing demand through population growth [31]. Back in the 1990s, global ecosystem services were valued in the order of US$33 trillion per year globally [32].

The real benefit of the so-called ecosystems approach[1] is that environmental impacts can be looked at in the round in terms of overall impacts on human wellbeing, asking whether the ecosystem services they provide outweigh any environmental harm that they may give rise to. For bioenergy cropping, the provision of energy is quite clearly a major benefit, which should be taken into account alongside environmental impacts. This is a much more holistic approach than environmental risk assessments.

However, there are limits. While there is a great deal of research into the valuation of ecosystem services in order to allow better weighting among different costs and benefits [34], such valuation is both difficult and contentious. This is because such values change between people, from place to place and over time.

A second problem is that knowledge of relationships between ecosystem services and the underlying ecosystem processes varies greatly. For provisioning services, including provisioning of food and bioenergy, plant growth results in crop yield, which is harvested and made available to people, subject to losses in the food chain. This is a simple, well quantified, linear relationship. By contrast, the relationships between pollinator numbers and crop yield is far less clear cut, while the aesthetic value given to agricultural landscapes varies over time [36]. While the ecosystem approach has a great deal of potential, the science base is not yet ready to use economic valuation of ecosystem services to determine the benefits of bioenergy projects.

[1] Several other environmental and sustainability analyses are similar to the ecosystems approach, in scope and intention, if not in method. The jargon can be quite confusing, not least because some words, e.g. sustainability, mean very different things to different people [33].

Table 2 Environmental performance of different energy crops across Europe

	Energy savings	Greenhouse effect	Acidification	Eutrophication	Summer smog	Ozone depletion	Human toxicity
Oil crops—FAME	+	0	0	–	0	–	0
Oil crops HVO	+	0	0	–	0	–	–
Oil crops Heat and power	+	0	–	–	0	–	–
Oil crops Heat	+	0	–	–	0	–	–
Oil crops Power	+	0	–	–	0	–	–
Woody crops—FT	+	0	0	0	0	0	0
Woody crops—2nd Generation Bioethanol	+	+	–	–	0	–	–
Woody crops—Heat and power	++	+	0	0	0	0	0
Woody crops—Heat	+	+	0	0	0	0	0
Woody crops—Power	+	0	0	0	0	0	0
Herb. Crops—FT	++	+	0	–	0	–	0
Herb. Crops—2nd generation Bioethanol	++	++	–	–	+	–	–
Herb. Crops—Heat and power	+++	++	0	–	0	–	0
Herb. Crops—Heat	++	++	–	–	0	–	–
Herb. Crops—Power	++	+	0	–	0	–	–
Sugar crops—1st generation Bioethanol	++	+	–	–	0	–	–

All values are given as inhabitat equivalents per 100 ha, i.e. per capita values across the European Union divided by yearly energy demand or average emissions, as appropriate: '+++': <−400; '++': −400 to −100; '+': −100 to −25; '0': −25 to +25; '−': 25 to 100; '− −': 100 to 400. Three forms of biodiesel are included; FAME = fatty acid methyl ester, the most common type, HVO = hydrogenated vegetable oil, FT = Fischer–Tropsch. Table from [29]

Table 3 Two classifications of ecosystem services, along with examples, including bioenergy, (A) as developed by the Millennium Ecosystem Assessment (MA) [35] and (B) by Fisher et al. [30]

Category	Provisioning services	Regulating services	Cultural services	Supporting services
(A)				
Description	Products obtained from ecosystems	Benefits obtained from regulation of ecosystem processes	Non-material benefits obtained from ecosystems	Services necessary for the production of all other ecosystem services
Example services	*Bioenergy*, food, fresh water, fuelwood, fibre, biochemicals, genetic resources	Climate regulation, disease regulation, water regulation, water purification, pollination	Spiritual and religious, recreation and ecotourism, aesthetic, inspirational, educational, sense of place, cultural heritage	Soil formation, nutrient cycling, primary production

Category	Intermediate services	Final services	Benefits
(B)			
Description	Services used indirectly by people	Services provided by ecosystems used by people	The final impact on human welfare, often produced in combination with other forms of capital, e.g. processing, knowledge, labour, infrastructure etc.
Example services	*Soil formation, primary production*	*Supply of plant biomass*	*Bioenergy*
	Nutrient cycling	Clean water provision	Drinking water
	Pollination	Food production	Fruit
	Soil formation, Primary production	Water regulation	Flood protection

The MA classification is useful to identify the services provided by a particular habitat, ecosystem or landscape, while Fisher et al.'s alternative is more suited to economic valuation of ecosystem services, as it distinguishes more clearly between the actual benefits enjoyed by people and the ecological processes required to deliver those outputs (B)

1.2.5 Post-Market Monitoring and Analysis

The approaches discussed above all assume some prior knowledge of the environmental risks; by definition, it is not possible to address unknown effects in advance. Therefore, there is a place for ongoing studies even once the crop has been introduced. Such studies are of two types; there is monitoring of the crop itself, and there is also ongoing environmental surveillance.

Whether a crop is monitored after its commercialisation depends on commercial, regulatory and research interests. Such monitoring can be as simple as a questionnaire for farmers.

There are many national and international programmes of environmental surveillance, looking at (for example) GHG inventories, biodiversity, water quality, agricultural and forestry statistics and land cover change. Such inventories address the environmental parameters of major policy interest, and should therefore reveal whether an environmental change, such as the introduction of bioenergy crops, is having, or has had, a major environmental impact. The problem is that it is often impossible to ascribe changes recorded by such surveillance to particular causes.

However, statistical analysis can identify plausible explanations. For example, it was suggested that the falling numbers of farmland birds observed in the 1970s and 1980s in the UK were due to agricultural intensification. This was tested by using multivariate techniques to relate 31 variables describing agricultural practices in England and Wales to bird numbers on farmland on an annual basis between 1962 and 1995. It was concluded that the large shifts in agricultural management between 1974 and 1991 gave a plausible explanation for the declines in farmland birds at this time [37]. Similarly, the reduction in diversity observed in wild plants in the UK during the late twentieth century [38] was more concentrated among those species that thrive in nutrient poor conditions, implying that increasing inputs of nitrogen into the environment was a potential cause; further analysis suggested that this was due largely to agriculture with a smaller component arising from atmospheric deposition from motor vehicles and industry [39].

To summarise, methods are in place for quantifying individual environmental impacts of individual bioenergy projects. However, it is difficult to quantify all major environmental impacts, not least because they are so sensitive to variation in environment and management. Far fewer data are needed to look at the environmental impacts of bioenergy relative to another land use, e.g. primary forest, agricultural fields or abandoned land. This is particularly true for biodiversity. Ecologists talk of enhanced or reduced biodiversity, more than absolute numbers of species or individuals. This is partly because biodiversity encompasses the diversity of genotypes, species and ecosystems, making absolute quantification of any ecosystem impossible; ecologists must use sampling techniques, and these tend to address the more visible taxa (higher plants, vertebrates, the larger invertebrates), and partly because any experimental evaluation entails comparisons. It is also because ecological impacts tend to be concerned with changes. Identical bioenergy crop systems would have a very different environmental

footprint if one has displaced species-rich primary forest, while the other has displaced abandoned, degraded agricultural land.

It is therefore necessary to identify potential environmental impacts, to prioritise on those particularly important (which may differ from place to place), to quantify them using indicators that are both locally appropriate and are consistent with national reporting frameworks [40], and compare them against some form of baseline or reference system.

2 Major Environmental Impacts of Bioenergy Crops

There is a very wide range of environmental impacts from any change in land use, and these vary greatly from place to place. Bioenergy cropping is no exception. Here the major impacts are discussed, but this list is far from exclusive and inevitably does not address detailed variation.

2.1 Competition for Land

The main ecosystem service provided by bioenergy cropping is the provisioning of fuel; the greater the energy yield of the fuel per unit hectare, the greater the ecosystem service. Yield varies between crops, soils, climates and management, with sugar cane, sugar beet and oil palm generating the highest yields of biofuel per unit area [41]. Evidence is accumulating that actual yields of ethanol and biodiesel from biofuel crops globally is typically less than has been reported on the basis of individual sites; there seems to be a yield gap between what can be achieved and what is often achieved in practice [41].

An important potential problem of growing bioenergy crops is that it might be at the expense of producing something else, in particular food. There is considerable concern that as the global need for food increases [42], agricultural land has been transferred from food to bioenergy production, which may result in indirect loss of primary forest for further food production [43]. It is not at all clear how such indirect impacts should be estimated [29].

In general, bioenergy crops that produce sugars give a high yield per unit area, and bioenergy from food crop species give low yields. Second generation crops are intermediate, but can be grown on land unsuitable for food production (Table 4) [44]. While there have been several attempts to analyse and model how much land might be available for bioenergy cropping (e.g. [45]), they, like all such models, have very high uncertainties associated with fluctuations in markets, technologies and policies. It is therefore unwise to rely on such forecasts for detailed planning; better simply to accept that competition between provisioning of food and of bioenergy threatens both food and energy security, and should be avoided wherever possible. One way is to utilise those elements of agro-ecosystems not

Table 4 Bioenergy crops ranked on the basis of land use and nitrogen intensity to harvest 1,000 GJ energy; there is much variation and uncertainty in these figures, which is why some have been ranked together even though the mean estimated values differ slightly [44]

	Land use intensity (ha/1000 GJ)	Rank	Nitrogen Intensity (kg N/1000 GJ)	Rank
Algae	0.3	1	1,100	11
Sugar Beet	1.9	2	460	8
Sugar Cane	2.3	2	110	2
Oil Palm	3.0	4	440	8
Miscanthus	4.2	5	210	5
Willow	5.3	6	90	1
Corn	4.9	6	490	8
Sweet Sorghum	6.1	8	390	7
Switchgrass	6.5	8	300	6
Birch	6.8	8	160	3
Poplar	7.2	8	160	3
Grain Sorghum	16.2	12	1,000	11
Rapeseed	16.5	12	1,400	12
Soybean	20.2	13	3,900	13

providing food, such as growing crops on degraded and abandoned land. This is not a global solution in itself; Cambpell et al. [46] estimate that less than 8% of the global energy demand can be generated this way. However, bioenergy can also be generated from mixed cropping systems, from crop, forest and urban wastes, either as biomass or biogas [43] (Table 1) and, potentially, from algae, that is highly productive per unit area (Table 4).

2.2 Climate Regulation

Bioenergy crops have the potential to improve the regulation of climate through reductions in GHG emissions, by drawing upon atmospheric carbon dioxide, as opposed to carbon dioxide that has been fossilised and buried for millions of years as coal, gas and oil. Whether or not such potential is being realised is a highly controversial issue. The two most important issues are the potential loss of carbon from soil and vegetation as a result of conversion of land to bioenergy cropping from other sources, and the GHG balance once the cropping system is established.

Carbon is stored in both soil and standing vegetation; the amount of carbon released during land use change depends on soil condition (the more the organic matter in the soil, the higher the level of carbon) and the previous land use (the greater the biomass of vegetation, the greater the carbon). Land clearance can result in influencing regional climate directly, and the subsequent oxidisation of

carbon in the soil can go on for over 50 years. This loss of carbon, the so-called carbon debt, needs to be repaid by GHG savings through the reduced use of fossil fuels before there is a net beneficial effect on climate regulation. The conversion of peaty tropical forests may take over 400 years of biofuel production before there is a net gain [47], Additional carbon debts can be incurred as the infrastructure and processing of the plant are constructed. By contrast, the use of degraded agricultural land carries little or no carbon debt, and the use of perennial crops is likely to result in carbon sequestration as the soils become richer in organic matter.

GHGs are released when the fuel is used to produce energy. They are also released during the manufacture and distribution of fertilisers and pesticides, during the cultivation of soil and other farming operations, and during the distribution of the crop. The greenhouse gases nitrous oxide (N_2O) and methane (CH_4) are much more potent than CO_2. N_2O has a CO_2 equivalent value (CO_2e) of 296 and can be released from the soil during cultivations, and by the manufacture of inorganic fertilisers. Those crops with a high nitrogen requirement, e.g. oil seed rape and maize, release more N_2O, potentially outweighing the benefits of savings in carbon [48]. Methane is 23 times as potent a greenhouse gas than CO_2, and is released during the breakdown of forage in the guts of livestock, and from their slurries and manures. Bioenergy production may reduce methane emissions, partly by using organic wastes (including livestock manures) as feedstocks for anaerobic digestion and potentially by bioenergy cropping replacing livestock as a use of lower grade agricultural land.

As stated above, the standard approach to estimate GHG emissions is life cycle analysis (LCA). For example, Adler et al. [49] used simulation models to compare GHG emissions for a range of bioenergy crops and cropping systems in Pennsylvania. They found that all systems provided net GHG savings, with N_2O production as the largest source of GHGs, and the displacement of fossil fuel providing the greatest sink of GHGs. The biggest savings were to be made by the second generation crops, switchgrass and poplar: these had the highest biomass yield, displacing more fossil fuel.

2.3 Nutrient Cycling

Food production entails the incorporation of nutrients by plants, to be passed on into the human diet. High levels of agricultural production have been achieved using chemical fertilisers, the manufacture of which is energy-intensive, releasing GHGs. Nitrogen and other nutrients may be lost as residues of the crop, as livestock manures and slurries and as food wastes. Such nutrients are either resources for future plant growth or potential sources of atmospheric pollution. Excess nutrients can be lost to the atmosphere and water courses, giving rise to GHG emissions and eutrophication. Soil disturbance can give rise to erosion and sediment loss and loss of phosphorus into watercourses, also resulting in eutrophication. Therefore, the environmental footprint of bioenergy crops is improved when

the use of chemical fertilisers is low, soil disturbance is avoided and the risk of losses of nitrogen and phosphorus are minimised.

Nutrient requirements for first generation crops grown for energy can differ from the equivalent food crops, as the desired levels of protein may differ, but they are still high compared with other bioenergy crops ([44]). SRC and *Miscanthus* have low nitrogen requirements per unit energy (Table 4) because they typically need fertilisation only during establishment, depending on soil conditions etc. Levels of nitrate leaching are high during soil disturbance during planting and grubbing up, but these are offset by low levels during the 15–30 years of growth and cultivation [50]. It is possible to plant SRC in locations which can use nitrogen leached from crops located upslope, thereby improving water quality. Sugar cane also has low requirements (Table 4), which may be reduced further as some varieties can obtain nitrogen from associated bacteria [51].

Plant residues, livestock manures and slurries, food wastes can be used as biofuel, and the digestates and ashes left after fuel production returned to the land, recycling some of the nutrients. It has been estimated that unused crop residues could account for over 3% of the EU's energy consumption [52], although the harvesting of residues over the medium term can reduce soil carbon. Also, there is scope for using treated sewage as a fertiliser of bioenergy crops because they do not enter the human food chain.

2.4 Water Footprint

Rising populations, increasing demands for agriculture and changing climates are all putting pressure on global freshwater supplies. Indeed, water shortages are more likely to limit global food production than land shortages in the coming decades [53]. The water footprints of bioenergy crops are therefore an essential component of their environmental impact.

Gerbens-Leenes et al. [54] recently reviewed the water footprints of major bioenergy crops on a national basis. Not surprisingly, different crops varied in how much water they required per unit of energy, with sugar beet, sugar cane and potato being particularly efficient. It takes a mere 1,400 L of water to produce 1 L of ethanol from sugar beet, compared with 9,800 L of water to produce the same amount of ethanol from sorghum [54]. These values are far higher than for fossil fuels and non-biological renewable energy sourses [55]. If crop yields are high, water footprints tend to be low, and vice versa [54]. There is a great deal of variation between countries (Fig. 4), and by implication, much more variation within countries.

Second generation crops also have very variable water footprints. Field trials indicate that *Miscanthus* can reduce drainage by over 30% compared with maize cropping in Illinois [56]; there is evidence that SRC takes more water than arable crops, and about the same as woodland, but the data are too limited to be conclusive [57]. Such results are hardly surprising; water utilisation is a function of

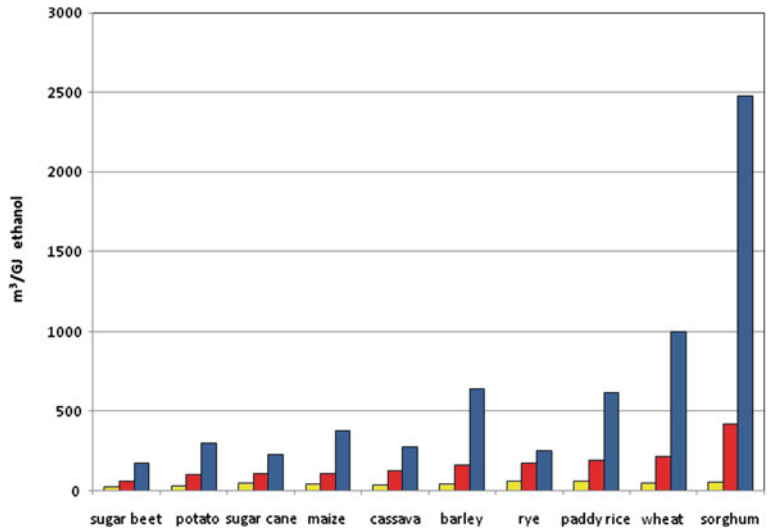

Fig. 4 Lowest national value (*light blue*), weighted-average global value (*mid-blue*) and highest national value (*dark blue*), of the water footprint for ten crops used to produce ethanol. Units in m3 of water required to produce 1 GJ of energy from ethanol. Redrawn from [54]

evapotranspiration, which in turn is a function of leaf area and length of growing season. Some plants found in arid areas, e.g. *Agave*, use crassulacean acid metabolism that makes them particularly efficient in water use and of potential value for bioenergy production [58].

Overall, the impact on water supplies depends on the previous land use, the scale of change and the choice of crops. A large scale switch from arable crops to perennial bioenergy crops is likely to result in increased water demand.

2.5 Biodiversity Conservation

The impacts of bioenergy cropping on biodiversity need to be considered at different scales: the biodiversity within the crop, the biodiversity of the landscape and catchment, and biodiversity at wider scales [13].

Biodiversity within the crop depends on the choice of crop and how it is managed as well as the environmental context. In general, the greater the structural complexity of the crop and more similar to pre-existing semi-natural or natural vegetation in the area, the greater the biodiversity.

The biodiversity of the first generation crops is similar to that of the same crops used for food or fodder, with differences due to different levels of fertilisers and, especially, herbicides and pesticides. The crop itself can have an impact on biodiversity, for example, if there is the potential for gene flow with wild relatives

[13]. It is also possible to design biodiverse plant assemblages as feedstocks, notably species-rich grass mixtures that can support invertebrates [7].

In temperate second-generation crops, the more complex the structure the greater the biodiversity, thus SRC willow stands hold higher densities of birds than SRC poplar, while mixed stands have higher densities still [59]. In the UK, SRC willow holds more individuals and more species of birds than arable and grassland fields in the same landscapes [61], while the denser and structurally simpler *Miscanthus* supported around the same numbers of birds as nearby grass and arable fields, albeit with a slightly different balance of species [62].

Weed control is often required to reduce competition from the ground vegetation with the crop. However, the ground flora is at the bottom of many ecological food chains, and hence strategies are needed to maintain productivity without removing this important food source for invertebrates, mammals and birds. In arable crops, this can be achieved using unsprayed areas [22] or selective herbicides [63]. In perennial crops, the most severe competition is during establishment, and ground vegetation may be allowed, or even encouraged, subsequently [59], although it will not thrive if the crop canopy is too dense. SRC plantations are often rich in invertebrates, but some of these are pests, which may result in the use of insecticides. This should be minimised so as not to impact the other species, and also to facilitate biological control of insect pests by natural enemies [59].

The same principles apply to bioenergy crops in the tropics; greater structural and temporal diversity enhances biodiversity as long as it mimics the conditions found by those species already in the landscape at least to some extent. As in temperate regions, much depends on what the bioenergy crop replaces, and the landscape in which it is placed. However, temperate landscapes have typically been highly transformed for agriculture, with the more biodiverse areas usually protected from further development. In the tropics, this process of transformation is still proceeding, with biodiversity being lost largely through loss of habitat but also through increased conflicts between human and conservation interests and the spread of invasive plants. Indeed, the potential for some second generation bioenergy crops to be invasive is, unfortunately, high [59]; trees of the genus Prosopis planted for biofuels have now invaded over 4 million ha in Africa, with serious consequences for biodiversity, as well as for water supplies and agriculture [60].

In Asia, the current generation of bioenergy crops are thought likely to increase pressure for land use change, and do not provide good quality habitat compared with land covers they are thought likely to replace [64]. Plantations of palm oil support only half the numbers of vertebrates as primary forest, and fewer than secondary forests, as they tend to be even-aged monoculture stands [65]. While bird species number can be improved by retaining an understory [66], the strategy of retaining forest fragments in plantations is less effective for conservation of biodiversity than the protection of whole forests [67].

At the landscape scale, the spatial arrangement of crops can make a difference to species richness and abundance. Biodiversity tends to be greatest at the edge of temperate second generation bioenergy crops, where they abut other habitats

including farmland, hedgerow or woodland. Therefore, biodiversity is increased by a mosaic of bioenergy crops and other land uses, with rotations and, ideally, a diversity of clones of bioenergy crops [59]. Such management brings diversity to the landscape which, in general, increases the biodiversity of agricultural landscapes [39, 68]. Thus, for example, the use of marginal land in mid-western USA for second generation bioenergy crops is thought likely to enhance bird species richness, while its use for arable bioenergy crops is likely to reduce bird diversity by reducing the diversity of habitats [69].

At wider scales, the concern is the loss of biodiverse habitats to make way for bioenergy cropping, directly or indirectly. Thus, the rapidly increasing areas of palm oil are accelerating deforestation and species loss in southeast Asia [70], and there are concerns that increased cultivation of sugarcane may lead to deforestation in Brazil [71].

3 Towards More Sustainable Bioenergy Cropping

Of the many potential environmental impacts of bioenergy production, two are particularly important at the global scale, one potentially positive and the other potentially negative. The positive impact is the replacement of fossil fuels by renewable energy, that should result in reduced GHG emissions, reducing the rate and severity of climate change [72]. The negative impact is that major expansion of cropped land to make way for this increased production may influence climate and water regimes, will be at the expense of biodiversity, landscape quality and cultural heritage, and may release a great deal of GHG emissions. The perfect bioenergy cropping system, therefore, is productive in net energy terms per unit area; has low requirements for water and nutrients across the life cycle; provides habitat for biodiversity; and can grow on low grade, carbon poor soils, and can therefore be grown on degraded and marginal land.

At the field and farm level, the environmental impacts will depend largely upon the choice of crop, what the crop will replace, crop management and the land use context. The most damaging direct impacts will typically result from the loss of carbon through disturbance of soils and pre-existing vegetation and the use of invasive bioenergy crops; indirect impacts arise if new land is brought into production. Benefits can be realised if the crops support native biodiversity, and are sited so as to intercept excess nutrients running from other areas of land. Delivering such benefits often requires taking landscape structure into account. Milder et al. [73] draw upon the ideas of landscape ecology to suggest guiding principles for obtaining environmental benefits from fitting bioenergy cropping into landscapes; they include promoting spatial heterogeneity and landscape connectivity to increase biodiversity and to make it easier for plants and animals to move between habitat patches; land suitability, including use of degraded land; low input, perennial and mixed species systems. These principles apply to different extents depending on the scale of farming [73], and they are most likely

to be successful if applied in partnership with local stakeholders [74] supported by knowledge exchange.

At larger scales, the major challenge is to address the trade-offs between bioenergy production and other forms of land use, and how to manage them using policies and other instruments. These need to be realistic about how much energy is likely to be produced without competing directly with food production or resulting in large-scale loss of primary forests. For example, it has been concluded that around 1.5 million hectares of *Miscanthus,* SRC willow and poplar could be grown in England and Wales on land less suitable for food production, yet this large area would only deliver 2.6% of UK's energy needs by 2020 [75]. As we have seen, there may only be enough abandoned and low-grade agricultural land available for biofuel crops to supply around 8% of current global energy demand [46]. Furthermore, the expansion of bioenergy cropping in some parts of the world may prove unsustainable under future climates [76]. Clearly, bioenergy cropping can only ever be part of the energy mix.

While bioenergy cropping has the potential to deliver environmental harm, it is becoming clear that it can deliver environmental and economic benefits. For the benefits to be delivered, bioenergy production systems should be analysed using different approaches according to the need, combing risk assessments, experiments, holistic analyses and monitoring. Furthermore, bioenergy cropping should be seen as part of a wider strategy for the efficient use of land, energy, food, water and natural resources, and such a strategy must address demand as well as supply, and needs to be tailored to the land management systems already in place.

Acknowledgments I would like to thank the Institute of Integrative and Comparative Biology for providing me the base for writing this chapter, and Evan DeLucia, Winnie Gerbens-Leenes and Jeff Wolt for permission to use figures from their publications.

References

1. Arnold JEM, Jongma J (1978) Fuelwood and charcoal in developing countries: an economic survey. Unasylva 29(118):2–9
2. Ladanai S, Vinterback J (2009) Global potential of sustainable biomass for energy. Report 013. Department of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala
3. Sorda G, Banse M, Kemfert C (2010) An overview of biofuel policies across the world. Energy Policy 38(11):6977–6988. doi:10.1016/j.enpol.2010.06.066
4. Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327(5967):812–818. doi:10.1126/science.1185383
5. Prochnow A, Heiermann M, Plochl M, Linke B, Idler C, Amon T, Hobbs PJ (2009) Bioenergy from permanent grassland - a review: 1. biogas. Bioresour Technol 100(21): 4931–4944. doi:10.1016/j.biortech.2009.05.070
6. Prochnow A, Heiermann M, Plochl M, Amon T, Hobbs PJ (2009) Bioenergy from permanent grassland - a review: 2. combustion. Bioresour Technol 100(21):4945–4954. doi:10.1016/j.biortech.2009.05.069

7. Tilman D, Hill J, Lehman C (2006) Carbon-negative biofuels from low-input high-diversity grassland biomass. Science 314(5805):1598–1600
8. Singh A, Nigam PS, Murphy JD (2011) Renewable fuels from algae: an answer to debatable land based fuels. Bioresour Technol 102(1):10–16. doi:10.1016/j.biortech.2010.06.032
9. IEA (2010) World total primary energy supply. IEA Energy Statistics. http://www.iea.org/stats/pdf_graphs/29TPES.pdf
10. Krebs JR, Wilson JD, Bradbury RB, Siriwardena GM (1999) The second silent spring? Nature 400(6745):611–612
11. Firbank LG, Heard MS, Woiwod IP, Hawes C, Haughton AJ, Champion GT, Scott RJ, Hill MO, Dewar AM, Squire GR, May MJ, Brooks DR, Bohan DA, Daniels RE, Osborne JL, Roy DB, Black HIJ, Rothery P, Perry JN (2003) An introduction to the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. J Appl Ecol 40(1):2–16
12. Perry JN, Rothery P, Clark SJ, Heard MS, Hawes C (2003) Design, analysis and statistical power of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. J Appl Ecol 40(1):17–31
13. Firbank LG (2008) Assessing the ecological impacts of bioenergy projects. Bioenerg Res 1(1):12–19
14. Haughton AJ, Bond AJ, Lovett AA, Dockerty T, Sunnenberg G, Clark SJ, Bohan DA, Sage RB, Mallott MD, Mallott VE, Cunningham MD, Riche AB, Shield IF, Finch JW, Turner MM, Karp A (2009) A novel, integrated approach to assessing social, economic and environmental implications of changing rural land-use: a case study of perennial biomass crops. J Appl Ecol 46(2):315–322. doi:10.1111/j.1365-2664.2009.01623.x
15. Firbank L, Lonsdale M, Poppy G (2005) Reassessing the environmental risks of GM crops. Nat Biotechnol 23(12):1475–1476
16. Qi A, Perry JN, Pidgeon JD, Haylock LA, Brooks DR (2008) Cost-efficacy in measuring farmland biodiversity - lessons from the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. Ann Appl Biol 152(1):93–101. doi:10.1111/j.1744-7348.2007.00193.x
17. Clark SJ, Rothery P, Perry JN, Heard MS (2007) Farm Scale Evaluations of herbicide-tolerant crops: assessment of within-field variation and sampling methodology for arable weeds. Weed Res 47(2):157–163
18. Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, Gray A, Olin SS, Schiemann J, Sears M, Wu F (2010) Problem formulation in the environmental risk assessment for genetically modified plants. Transgenic Res 19(3):425–436. doi:10.1007/s11248-009-9321-9
19. Gibbons DW, Bohan DA, Rothery P, Stuart RC, Haughton AJ, Scott RJ, Wilson JD, Perry JN, Clark SJ, Dawson RJG, Firbank LG (2006) Weed seed resources for birds in fields with contrasting conventional and genetically modified herbicide-tolerant crops. Proc R Soc B Biol Sci 273(1596):1921–1928
20. Butler SJ, Vickery JA, Norris K (2007) Farmland biodiversity and the footprint of agriculture. Science 315(5810):381–384
21. Storkey J, Bohan DA, Haughton AJ, Champion GT, Perry JN, Poppy GM, Woiwod IP (2008) Providing the evidence base for environmental risk assessments of novel farm management practices. Environ Sci Policy 11(7):579–587. doi:10.1016/j.envsci.2008.06.001
22. Pidgeon JD, May MJ, Perry JN, Poppy GM (2007) Mitigation of indirect environmental effects of GM crops. Proc R Soc B Biol Sci 274(1617):1475–1479. doi:10.1098/rspb.2007.0401
23. Wilkinson MJ, Davenport IJ, Charters YM, Jones AE, Allainguillaume J, Butler HT, Mason DC, Raybould AF (2000) A direct regional scale estimate of transgene movement from genetically modified oilseed rape to its wild progenitors. Mol Ecol 9(7):983–991
24. Sears MK, Hellmich RL, Stanley-Horn DE, Oberhauser KS, Pleasants JM, Mattila HR, Siegfried BD, Dively GP (2001) Impact of Bt corn pollen on monarch butterfly populations: a risk assessment. Proc Natl Acad Sci U S A 98(21):11937–11942
25. EU (2009) Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC European Union, Brussels

26. Davis SC, Anderson-Teixeira KJ, DeLucia EH (2009) Life-cycle analysis and the ecology of biofuels. Trends Plant Sci 14(3):140–146. doi:10.1016/j.tplants.2008.12.006
27. Zah R, Faist M, Reinhard J, Birchmeier D (2009) Standardized and simplified life-cycle assessment (LCA) as a driver for more sustainable biofuels. J Clean Prod 17:S102–S105. doi:10.1016/j.jclepro.2009.04.004
28. Chiaramonti D, Recchia L (2010) Is life cycle assessment (LCA) a suitable method for quantitative CO2 saving estimations? the impact of field input on the LCA results for a pure vegetable oil chain. Biomass Bioenerg 34(5):787–797. doi:10.1016/j.biombioe.2010.01.022
29. Rettenmaier N, Koppen S, Gartner SO, Reinhardt GA (2010) Life cycle assessment of selected future energy crops for Europe. Biofuels Bioprod Bioref 4(6):620–636. doi:10.1002/bbb.245
30. Fisher B, Turner RK (2008) Ecosystem services: classification for valuation. Biol Conserv 141(5):1167–1169. doi:10.1016/j.biocon.2008.02.019
31. Millennium Ecosystem Assessment (2005) Synthesis report. Island Press, Washington
32. Costanza R, d'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, Oneill RV, Paruelo J, Raskin RG, Sutton P, van den Belt M (1997) The value of the world's ecosystem services and natural capital. Nature 387(6630):253–260
33. Buchholz T, Luzadis VA, Volk TA (2009) Sustainability criteria for bioenergy systems: results from an expert survey. J Clean Prod 17:S86–S98. doi:10.1016/j.jclepro.2009.04.015
34. Kumar P (ed) (2010) The economics of ecosystems and biodiversity: ecological and economic foundations. Earthscan, London
35. Millennium Ecosystem Assessment (2003) Ecosystems and human well being: a framework for assessment. Island Press, Washington
36. Firbank LG, Bradbury RB, McCracken DI, Stoate C (2011) Enclosed farmland. In: UK National Ecosystem Assessment: technical report. UNEP- WCMC, Cambridge, pp 197–240
37. Chamberlain DE, Fuller RJ, Bunce RGH, Duckworth JC, Shrubb M (2000) Changes in the abundance of farmland birds in relation to the timing of agricultural intensification in England and Wales. J Appl Ecol 37(5):771–788
38. Preston CD, Pearman DA, Dines TD (2002) New atlas of the British and Irish flora. Oxford University Press, Oxford
39. Firbank LG, Petit S, Smart S, Blain A, Fuller RJ (2008) Assessing the impacts of agricultural intensification on biodiversity: a British perspective. Philos Trans R Soc B Biol Sci 363(1492):777–787. doi:10.1098/rstb.2007.2183
40. Lattimore B, Smith CT, Titus BD, Stupak I, Egnell G (2009) Environmental factors in woodfuel production: opportunities, risks, and criteria and indicators for sustainable practices. Biomass Bioenerg 33(10):1321–1342. doi:10.1016/j.biombioe.2009.06.005
41. Johnston M, Foley JA, Holloway T, Kucharik C, Monfreda C (2009) Resetting global expectations from agricultural biofuels. Environ Res Lett 4(1):1–9. doi:01400410.1088/1748-9326/4/1/014004
42. Foresight (2011) The future of food and farming: challenges and choices for global sustainability. The Government Office for Science, London
43. Tilman D, Socolow R, Foley JA, Hill J, Larson E, Lynd L, Pacala S, Reilly J, Searchinger T, Somerville C, Williams R (2009) Beneficial biofuels-the food, energy, and environment trilemma. Science 325(5938):270–271. doi:10.1126/science.1177970
44. Miller SA (2010) Minimizing land use and nitrogen intensity of bioenergy. Environ Sci Technol 44(10):3932–3939. doi:10.1021/es902405a
45. Lovett AA, Sunnenberg GM, Richter GM, Dailey AG, Riche AB, Karp A (2009) Land use implications of increased biomass production identified by GIS-based suitability and yield mapping for Miscanthus in England. Bioenerg Res 2(1–2):17–28. doi:10.1007/s12155-008-9030-x
46. Campbell JE, Lobell DB, Genova RC, Field CB (2008) The global potential of bioenergy on abandoned agriculture lands. Environ Sci Technol 42(15):5791–5794. doi:10.1021/es800052w
47. Fargione J, Hill J, Tilman D, Polasky S, Hawthorne P (2008) Land clearing and the biofuel carbon debt. Science 319(5867):1235–1238. doi:10.1126/science.1152747

48. Crutzen PJ, Mosier AR, Smith KA, Winiwarter W (2008) N2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. Atmos Chem Phys 8(2):389–395
49. Adler PR, Del Grosso SJ, Parton WJ (2007) Life-cycle assessment of net greenhouse-gas flux for bioenergy cropping systems. Ecol Appl 17(3):675–691
50. Goodlass G, Green M, Hilton B, McDonough S (2007) Nitrate leaching from short-rotation coppice. Soil Use Manag 23(2):178–184. doi:10.1111/j.1475-2743.2006.00080.x
51. Momose A, Ohtake N, Sueyoshi K, Sato T, Nakanishi Y, Akao S, Ohyama T (2009) Nitrogen fixation and translocation in young sugarcane (Saccharum officinarum L.) plants associated with endophytic nitrogen-fixing bacteria. Microbes Environ 24(3):224–230. doi:10.1264/jsme2.ME09105
52. Scarlat N, Martinov M, Dallemand J-F (2010) Assessment of the availability of agricultural crop residues in the European Union: Potential and limitations for bioenergy use. Waste Manag 30(10):1889–1897. doi:10.1016/j.wasman.2010.04.016
53. Hanjra MA, Qureshi ME (2010) Global water crisis and future food security in an era of climate change. Food Policy 35(5):365–377. doi:10.1016/j.foodpol.2010.05.006
54. Gerbens-Leenes W, Hoekstra AY, van der Meer TH (2009) The water footprint of bioenergy. Proc Natl Acad Sci U S A 106(25):10219–10223. doi:10.1073/pnas.0812619106
55. Gerbens-Leenes PW, Hoekstra AY, van der Meer T (2009) The water footprint of energy from biomass: A quantitative assessment and consequences of an increasing share of bio-energy in energy supply. Ecol Econ 68(4):1052–1060. doi:10.1016/j.ecolecon.2008.07.013
56. McIsaac GF, David MB, Mitchell CA (2010) Miscanthus and switchgrass production in central illinois: impacts on hydrology and inorganic nitrogen leaching. J Environ Qual 39(5):1790–1799. doi:10.2134/jeq2009.0497
57. Dimitriou I, Busch G, Jacobs S, Schmidt-Walter P, Lamersdorf N (2009) A review of the impacts of short rotation coppice cultivation on water issues. Landbauforsch Volk 59(3):197–206
58. Somerville C, Youngs H, Taylor C, Davis SC, Long SP (2010) Feedstocks for lignocellulosic biofuels. Science 329(5993):790–792. doi:10.1126/science.1189268
59. Dauber J, Jones MB, Stout JC (2010) The impact of biomass crop cultivation on temperate biodiversity. GCB Bioenerg 2(6):289–309. doi:10.1111/j.1757-1707.2010.01058.x
60. Witt ABR (2010) Biofuels and invasive species from an African perspective - a review. GCB Bioenerg 2(6):321–329. doi:10.1111/j.1757-1707.2010.01063.x
61. Sage R, Cunningham M, Boatman N (2006) Birds in willow short-rotation coppice compared to other arable crops in central England and a review of bird census data from energy crops in the UK. Ibis 148(s1):184–197
62. Sage R, Cunningham M, Haughton AJ, Mallott MD, Bohan DA, Riche A, Karp A (2010) The environmental impacts of biomass crops: use by birds of Miscanthus in summer and winter in Southwestern England. Ibis 152(3):487–499
63. Sotherton NW (1991) Conservation headlands: a practical combination of intensive cereal farming and conservation. In: Firbank LG, Carter N, Darbyshire JF, Potts GR (eds) The ecology of temperate cereal fields, 32nd symposium of the British Ecological Society. Blackwell Scientific Publications, Oxford, pp 373–397
64. Phalan B (2009) The social and environmental impacts of biofuels in Asia: an overview. Appl Energy 86:S21–S29. doi:10.1016/j.apenergy.2009.04.046
65. Fitzherbert EB, Struebig MJ, Morel A, Danielsen F, Bruhl CA, Donald PF, Phalan B (2008) How will oil palm expansion affect biodiversity? Trends Ecol Evol 23(10):538–545. doi:10.1016/j.tree.2008.06.012
66. Najera A, Simonetti JA (2010) Can oil palm plantations become bird friendly? Agrofor Syst 80(2):203–209. doi:10.1007/s10457-010-9278-y
67. Edwards DP, Hodgson JA, Hamer KC, Mitchell SL, Ahmad AH, Cornell SJ, Wilcove DS (2010) Wildlife-friendly oil palm plantations fail to protect biodiversity effectively. Conserv Lett 3(4):236–242. doi:10.1111/j.1755-263X.2010.00107.x
68. Benton TG, Vickery JA, Wilson JD (2003) Farmland biodiversity: is habitat heterogeneity the key? Trends Ecol Evol 18(4):182–188

69. Meehan TD, Hurlbert AH, Gratton C (2010) Bird communities in future bioenergy landscapes of the upper midwest. Proc Natl Acad Sci U S A 107(43):18533–18538. doi:10.1073/pnas.1008475107
70. Struebig MJ (2010) Reassessing the "real scenario" regarding the environmental sustainability of palm oil. Renew Sustain Energy Rev 14(8):2443–2444. doi:10.1016/j.rser.2010.02.016
71. Martinelli LA, Filoso S (2008) Expansion of sugarcane ethanol production in Brazil: environmental and social challenges. Ecol Appl 18(4):885–898
72. Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (eds) (2007) Contribution of working group II to the fourth assessment report of the intergovernmental panel on climate change, 2007. Cambridge University Press, Cambridge
73. Milder JC, McNeely JA, Shames SA, Scherr SJ (2008) Biofuels and ecoagriculture: can bioenergy production enhance landscape-scale ecosystem conservation and rural livelihoods? Int J Agric Sustain 6(2):105–121. doi:10.3763/ijas.2008.0344
74. Atwell RC, Schulte LA, Westphal LM (2010) How to build multifunctional agricultural landscapes in the US Corn Belt: add perennials and partnerships. Land Use Policy 27(4):1082–1090. doi:10.1016/j.landusepol.2010.02.004
75. Bauen AW, Dunnett AJ, Richter GM, Dailey AG, Aylott M, Casella E, Taylor G (2010) Modelling supply and demand of bioenergy from short rotation coppice and Miscanthus in the UK. Bioresour Technol 101(21):8132–8143. doi:10.1016/j.biortech.2010.05.002
76. Bryan BA, King D, Wang EL (2010) Biofuels agriculture: landscape-scale trade-offs between fuel, economics, carbon, energy, food, and fiber. GCB Bioenerg 2(6):330–345. doi:10.1111/j.1757-1707.2010.01056.x

Key Issues in Life Cycle Assessment of Biofuels

Anoop Singh and Stig I. Olsen

Abstract The production of sustainable bioenergy is a challenging task in the promotion of biofuels for replacing the fossil based fuels to get cleaner environment and also to reduce the dependency on other countries and uncertainty of fuel price. One biofuels might be very attractive for heat production and not so attractive for electricity and transport purposes. The commercial-scale production of biofuels requires careful consideration of several issues that can be broadly categorized as: selecting biomass, cultivation technology, growth inputs (nutrients, water, etc.) and biofuel conversion technology. The life cycle assessment (LCA) is a tool that can be used effectively in assessing various biofuels for their sustainability and can help to policy makers to choose the best biofuels for specific purpose. Allocation method is very important in assessing the sustainability of biofuels as different allocation methods responded differently. The present chapter is an effort to highlight the key issues to consider in conducting an LCA for biofuels.

1 Introduction

The increasing industrialization and motorization of the world has led to a steep rise for the energy demand, especially fossil fuels [1]. Global total primary energy supply has almost doubled since 1974, rising to 1,200 mtoe in 2007 in which fossil fuels accounted 80% share including 34% oil alone [27]. The global CO_2 emissions from the fuel consumption have increased by 88% since 1974 and rose to 29.4 Gt of CO_2 in

A. Singh (✉) · S. I. Olsen
Quantitative Sustainability Assessment, Department of Management Engineering, Technical University of Denmark, Lyngby, Denmark
e-mail: apsinghenv@gmail.com

K. Gopalakrishnan et al. (eds.), *Sustainable Bioenergy and Bioproducts*, Green Energy and Technology, DOI: 10.1007/978-1-4471-2324-8_11,

2008, of which 22% is emitted by transport sector [28]. The fossil fuel reserves are becoming exhausted and found major greenhouse gas (GHG) emissions contributor on the consumption of fossil fuels to fulfill the energy demand [42, 50, 58, 59, 73] that leads to various environmental challenges including climate change, receding of glaciers, rise in sea level, loss of biodiversity, etc. [21]. Increasing energy demand leads to an increase in crude oil price that directly affected to global economic activity [23]. A worrying statistic is that global production of oil and gas is approaching its maximum and the world is now finding one new barrel of oil for every four it consumes [2]. All these concerns related to energy security, environment and sustainability have led to a move toward alternative, renewable, sustainable, efficient and cost-effective energy sources with lesser emissions.

Renewable energy can play a crucial role in dealing with energy security, eco-friendliness and climate change issues at global and national levels [50, 51, 62–64, 70]. Renewable and carbon neutral biofuels are necessary for environmental and economic sustainability. Hence, as an alternative to fossil fuels, biofuels have been portrayed as a future leading supplier of energy sources that have the ability to increase the security of supply, reduce the vehicle emissions and provide a steady income for farmers.

The life cycle assessment (LCA) of biofuels is the key to observe their sustainability. There is a need to conduct LCA of biofuels production system on the basis of their local conditions, as one biofuels cannot be sustainable for all geographic locations, due to variations in resource availability, climate, environmental, economical and social conditions, policies, etc. Therefore, LCA can be used as a tool to assess the sustainability of various biofuels for different locations. Various LCA studies varies in defining the various criteria, such as, scope and goal, system boundary, reference system, allocation method, etc. The present chapter is an effort to highlight the key issues that could be kept in mind to conduct an LCA of biofuel production system to get a more holistic perspective of their sustainability.

2 Necessity of Biofuels

The fossil fuels accounted major share in the global primary energy consumption and the fossil fuels use is now widely unaccepted as sustainable fuel due to depleting resources and higher emission of GHGs in the environment that already have exceeded the "dangerously high" threshold of 450 ppm CO_2e [56]. This leads to develop abatement techniques and adopt policies to promote those renewable energy sources which are capable in sequestering the atmospheric CO_2, to minimize the dependency on fossil reserves and also to maintain environmental and economic sustainability [5, 50, 51, 63, 64]. The recent research activities are intensively focused on renewable fuels in order to fulfill the increasing energy demand and to reduce the fossil fuels consumption and dependency either in order to provide local energetic resources and or as a means for reducing GHG emissions to reduce the climate change effects [60, 61].

The biomass-based fuels can be a possible solution for all these issues related to energy production and consumption, as they are renewable by nature, and there are possibilities to use them for heat, electricity and transportation fuel, with little change to current technologies and have significant potential to improve sustainability and reduce GHG emissions [7]. An increasing number of developed and developing countries found biofuels as a key to reducing reliance on foreign oil, lowering GHG emissions and meeting rural development goals [16, 48]. Several biofuels were proposed to displace fossil fuels in order to eliminate the vulnerability of energy sector [37, 45, 50, 51, 63, 64]. Research on improving biofuels production has been accelerating for both ecological and economical reasons, primarily for its use as an alternative to petroleum-based fuels [50]. In recent years, the use of liquid biofuels in the transport sector has shown rapid global growth, driven mostly by policies focused on achievement of energy security, and mitigation of GHG emissions [26].

3 Importance of Sustainability

Sustainable development can be defined as, the fulfillment through the optimal use of any available source within a production system. Energy conversion, utilization and access underlie many of the great challenges associated with sustainability, environmental quality, security and poverty [37, 38]. World Commission on Environment and Development defined the term 'sustainability' as "the development that meets the needs of the present without compromising the ability of future generations to meet their own needs" [66]. Sustainability assessment of products or technologies is normally seen as encompassing impacts in three dimensions i.e. social, environmental and economic [13]. These three dimensions form the backbone of sustainability standards. To replace the fossil fuels with biofuels, there is a need to maximize the environmental and social value of biofuels that is also important for the future of biofuels industry and market potential depends on being cost competitive with fossil fuels (Fig. 1). These three interrelated goals must stay in balance for biofuels to remain sustainable.

The sustainability of biofuels production depends on the net energy gain fixed in the biofuels that depends on the production process parameters, such as land type where the biomass is produced, the amount of energy-intensive inputs and the energy input for harvest, transport and running the processing facilities [22], emissions and their production cost. The most used indicators to measure the biofuels sustainability includes life cycle energy balance, quantity of fossil energy substituted per hectare, co-product energy allocation, life cycle carbon balance and changes in soil utilization [57].

Gnansounou [18] suggested that due to the multi-dimensional impact of biofuels, the sustainability impact assessment of policies is as relevant as the sustainability assessment of production pathways and regulatory impact assessment. Sustainability

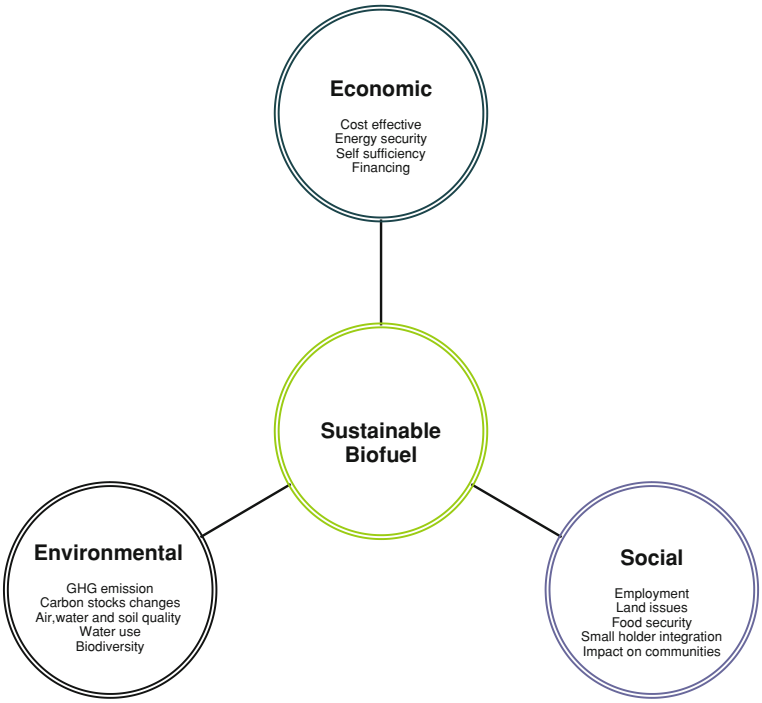

Fig. 1 Economic, social and environmental aspects of sustainable biofuels (Adopted from [29])

evaluation of biofuels is a multicriterial problem; the main indicators for a sustainable biofuels are pointed out by Silva Lora et al. [57] as follows:

- To be carbon neutral.
- Not to affect the quality, quantity and rational use of available natural resources.
- Not to have undesirable social consequences.
- To contribute to the societal economic development and equity.
- Not to affect the biodiversity.

The major factors that will determine the impacts of biofuels include their contribution to land-use change, the feedstock used, and technology use and scale of production. In order to ensure net societal benefits of biofuels production, governments, researchers and companies will need to work together to carry out comprehensive assessments, map suitable and unsuitable areas, and define and apply standards relevant to the different circumstances of each country [46]. The sustainable biofuel production is directed by environmental impacts (direct and indirect), economic viability including societal and political acceptance. Yan and Lin [69] revealed that the interactions among various sustainability issues make the assessment of biofuel development difficult and complicated. The complexity during the whole biofuel production chain generates significantly different results

due to the differences in input data, methodologies applied, and local geographical conditions. The LCA can be used as an effective tool for addressing environmental sustainability issues.

4 Life Cycle Assessment

LCA is a tool to assess the environmental impacts and resources used throughout a product's life cycle and consider all attributes or aspects of natural environment, human health and resources [37] and can be defined as a method for analyzing and assessing environmental impacts of a material, product or service along its entire life cycle [32]. The complete life cycle of the biofuels includes each and every step from raw material production and extraction, processing, transportation, manufacturing, storage, distribution and utilization of the biofuel. In addition, the life cycle stages can have harmful effects or benefits of different environmental, economical and social dimensions, due to these facts; the complete fuel chains from different perspectives is of crucial importance in order to achieve sustainable biofuels [40].

The LCA methodology can be useful to acquire a comprehensive knowledge of the environmental impacts generated by industrial products during their whole life cycle [10]. LCA has been the method of choice in recent years for various kinds of new technologies for bioenergy and carbon sequestration. LCA is a universally accepted approach of determining the environmental consequences of a particular product over its entire production cycle [44].

Various scientists have employed LCA on biofuel production systems [4, 17, 19, 36, 54, 72] and some useful results considering the factors (e.g. biomass, technologies, use, system boundary, allocation, reference system, etc.) affecting the outcome of the analysis have been obtained [63]. Gnansounou et al. [19] also stated that monitoring reduction of GHG emissions and estimations of substitutional efficiency with respect to fossil fuels is subject to significant uncertainty and inaccuracy associated with the LCA approach.

Reinhard and Zah [54] distinguished the two main approaches of LCA, i.e. the attributional and the consequential approach; both approaches differ with respect to system delimitation and the use of average versus marginal data. Attributional LCA describes the environmentally relevant physical flows to and from a life cycle and its subsystems, while consequential LCA describes how environmentally relevant flows will change in response to possible decisions [12]. Attributional LCA is limited to a single full life cycle from cradle to grave and consequential LCA is not limited to one life cycle, it uses system enlargement to include the life cycles of the products affected by a change of the physical flows in the central life cycle. The co-production has to be treated by applying allocation factors in attributional LCA, while in consequential LCA allocation is avoided as marginal data is used instead of average data in attributional LCA. Marginal data is represented by the product, resource, supplier or technology, which is the most

Table 1 Main problems associated with an LCA study (Adopted from [52])

LCA steps	Problem
Goal and scope	• Functional unit
	• Boundary selection
	• Social and economic impacts
	• Alternative scenario (reference system) considerations
Inventory analysis	• Allocation
	• Negligible contribution ('cutoff') criteria
	• Local technical uniqueness
Impact assessment	• Impact category and methodology selection
	• Spatial variation
	• Local environmental uniqueness
	• Dynamics of the environment
	• Time horizons
Interpretation	• Weighting and valuation
	• Uncertainty in the decision process
All	• Data availability and quality

sensitive to changes in demand and economic value criteria are used to identify the marginal products [12, 54].

Zah et al. [71] suggested two assessment steps to conduct a LCA of a biofuel, i.e. the modeling of the life cycle inventory (LCI) by quantifying all relevant flows of materials and energy along the value chain and the environmental impact assessment that evaluates the impacts of all materials and energy flows. For an LCA of biofuel, cultivation is the most relevant step followed by the processing of the biofuel, and transport of the fuel is of minor importance [71, 72].

LCA of biofuel production system requires a careful design regarding the goal and scope definition, choice of functional unit, reference system, system boundaries and appropriate inventory establishment and allocation of emissions in products and byproducts. The main problems associated with an LCA study is summarized in Table 1. Larson [39] describes four input parameters to cause the greatest variation and uncertainties in LCA results of biofuel production, namely: climate-active plant species (species with ability or otherwise to adapt to climate change); assumptions about N_2O emissions; the allocation method for co-product credits; and soil carbon dynamics.

4.1 Goal

The first step of LCA is to define the goal of the study. The goal is very important as the method depends on the goal which can influence the result of the study and provide an incorrect conclusion. The goal should unambiguously state the proposed application of the results, including the reasons for conducting the study and

the intended audience (to whom results are intended to be communicated). Wenzel et al. [67] stated that goal definition should answer the following three questions:

1. For what will the results of the LCA be used?
2. What decisions can be made on the basis of the LCA?
3. What is the nature and extent of these decisions?

The main goal for LCA of biofuels should be to evaluate the environmental impacts of the system and to quantify the ecological benefits from the replacement of the conventional or reference system. The results of such study can be utilized by policy maker and consumers to determine the optimum eco-friendly biofuel [63].

For an LCA study of biofuels, the goal can be defined as; the goal of the LCA is to identify options for improving the environmental performance of biofuels. The results of the study will be used to develop best process for biofuel production. The industrialist and policy maker will be able to analyze the effects of replacing the fossil-based fuels, changes in their processes (technology), inputs and product composition on the total environmental impact. The information generated from the study, can be used to prioritize different measures for promotion of biofuels that can improve the environmental performance from energy consumption.

4.2 Scope

This step establishes the main characteristics of an intended LCA study and can be defined as temporal, geographical and technology coverage, coverage of economic processes, coverage of environmental interventions and impact categories, the mode of analysis employed and the overall level of sophistication of the study in relation to its goal. The scope should be sufficiently well defined to ensure that the breadth, the depth and the detail of the study are compatible and sufficient to address the stated goal. LCA is an iterative technique, therefore, scope may need to be modified while the study is being conducted due to collection of additional information [20].

For a study of algal bioethanol production in Denmark, the scope can be described as to identify processes those requires improvement to make the production system more sustainable (economically and environmentally) to replace the fossil-based fuels. The data used in the study is representative of the present state of technology in Denmark. The study uses the most recent data available from 1990 to 2010. The study time is 12 person-months and a large proportion of that is devoted to the collection of representative data of the most important cultivation, harvesting, ethanol production, recycling of waste and upgrading processes.

4.3 Functional Unit

The functional unit describes the primary function(s) fulfilled by a product system, and indicates how much of this function is to be considered in the intended LCA study [20]. The functional unit depends on the goal of the study and for biofuels studies must be expressed in terms of per unit output (i.e. kWh or km) basis. Functional unit must be defined as closely as possible to the end-use of the product and must be in SI system. For transport services the functional unit ought to be expressed in 'per km distance travelled' (per tkm or per pkm) and should not be expressed in 'unit energy at fuel tank' (per MJ); as mechanical efficiency varies from one fuel to another and from one engine type to another [19, 63] and for residential or industrial services, functional unit could be 1 kWh electricity or heat.

4.4 System Boundary

Different system boundaries among various studies of biofuel production have caused considerable variation in LCA estimates since they vary not only according to start and end points (e.g. well to tank and well to wheel) but also over space and time in a way that can dramatically affect energy and GHG balances [9]. A uniform and clear determination of system boundaries should accurately estimate the possible environmental impacts other than GHG emissions between LCA for biofuels and conventional fuels [15]. Initial boundaries of the system are determined by the goal and the scope of the analysis and inputs/outputs in the unit processes are linked within the boundaries of the system [63].

The main ambition of most LCA study of biofuels is comparing biofuels with their fossil substitutes. The utilization stage is very crucial because the final energy produced from tank for a given end-use (transport/heat/electricity) depends on the combustion performances of that engine using that fuel [19, 49, 64]. Researchers used different system boundaries to compare environmental impact of biofuels with fossil fuels, e.g. the 'well to tank' system [14, 41], and 'well to wheel' or 'cradle to grave' system [49, 65]. Inconsistency of system boundaries in LCA analysis of biofuel production system through omission of the production of various inputs and utilization could cause a significant variation on the results of LCA study. To understand and manage the energy efficiency of renewable energy sources and related GHG emissions, the whole system should be considered [47].

Ekvall and Weidema [12] concluded in the "system boundaries and input data in consequential LCI analysis" study that the boundaries of the system investigated should ideally be defined at the point where the consequences are so small, or the uncertainties so large, that further expansion of the boundaries will yield no information that is significant for any realistic decision. In addition to the system enlargements caused by displacement, the system must include the consequences induced by co-products [54]. The consequences driven by co-products stemming

from multifunctional processes are important to consider them in defining the system boundaries. Ekvall and Weidema [12] have defined rules for system enlargement driven by multifunctional processes.

The 'well to tank' approach is sufficient only for comparing various technologies for biofuel production, while 'well to wheel' (cradle to grave) is the best approach for comparing utilization among different biofuels and/or with fossil fuels. System enlargement by multifunctional processes can avoid consequences caused by co-products of biofuel production system.

4.5 Reference System

The choice of reference system influences the results of LCA study; therefore it is important to choose an identical reference system to the conventional system which is targeted to replace with biofuels. The goal of the study determines the choice of the reference system, e.g. whether biofuel is intended to replace conventional transport fuel or coal for heat and electricity. A detailed description and impact analysis of the reference system is mandatory for comparing the results.

System analysis is possible by comparing the biofuel system with a targeted (conventional) reference system which is limited to a fossil fuel system, in most of the biofuel's LCA studies. In some cases misconceptions have been noticed, as in the case when co-products from the biofuel production system replace an existing product whose GHG balance is significantly different. In such case, a reference substituted product should be defined. The same applies to the case when the production of feedstock for biofuels uses land that was previously storing carbon such as forests or grasslands. In this case, a "previous land use" baseline should be taken under consideration for the determination of carbon emissions due to land-use change [19]. The fossil reference system that used for the comparison of results obtained in LCA of biofuels also produces the same products/services from fossil reference system.

4.6 Inventory Analysis

In the Inventory analysis step, the product system(s) is defined, which includes setting the system boundaries, designing the flow diagrams with unit processes, collecting the data for each processes, performing allocation steps for multifunctional processes and completing the final calculations [20]. A LCI is a process of quantifying energy and raw material requirements, environmental pollution for the entire life cycle of a product, process, or activity [55]. The inventory analysis requires data on the physical inputs and outputs of the processes of the product system, regarding product flows as well as elementary flows. Such data are mostly collected on a case-by-case basis, with the help of the companies involved.

In addition, there are a number of public data bases, which are used more generally, e.g. ecoinvent database, ELCD database, ETH database, BUWAL/SAEFL database, etc. [20]. The outcome of the inventory analysis is the inventory table, which is used in the impact assessment as an input.

LCA studies based on non-local inventory data may show inaccurate results, basically due to the potential differences in the environmental performance of the regions [6]. In order to account for these potential differences and apply inventory data in the form most suitable to the local conditions, de Eicker et al. [10] has summarized the following characteristics to generate an inventory database identical to local conditions:

- All processes must be addressed individually, as unit processes of the life cycle of the product, instead of a cumulative LCI where all steps are aggregated into one single dataset.
- All datasets should be clearly documented and include all details, in order to have a maximum transparency in the database.
- The database should provide different alternatives for the same product, related to technological variations.

The inventory analysis requires very extensive data. The outcome of the study totally depends on the availability and quality of the datasets. So that, there is a great need to collection of standardized data, especially for background processes. The main steps of inventory analysis include data collection and estimations, validation of data and relating data to the specific processes within the system boundaries. After the initial data collection, of which the source should be clearly declared, the system boundaries can be refined as a result of decisions on exclusion of sub-systems, exclusion of material flows or inclusion of new unit processes [63]. The validation of data as a mean of data quality improvement or the need for supplementary data would improve the outcome of the analysis [34]. In support of that statement, Monti et al. [41] mentioned that external data from the scientific literature should be obtained for inventory enhancement and accurate representation of the system.

4.7 Impact Assessment

Onn and Yusoff [43] defined life cycle impact assessment (LCIA) is a method used to derive the environmental burdens from selected stages of the product or service. LCIA is structured in classification, characterization, normalization and weighting. Impact assessment establishes a relationship between the product or process and its potential impacts on human health, environment and sources depletion [55]. ISO developed a standard for conducting an impact assessment entitled ISO 14042, LCIA [31]. The first three steps (i.e. selection and definition of impact categories, classification and characterization among others) are mandatory steps for the determination of impact categories. Each impact category corresponds to an

important environmental problem (e.g. eutrophication, depletion of non renewable energy resources, ozone depletion, etc.). Characterization sorted all inventory data into different impact categories according to their effect on the environment. The inventory data within each category will be multiplied by a characterization factor to differentiate the substances according to their severity as substances may have varied severity. Normalization enables assessing the relative contribution of the product's impacts to overall contribution of existing impacts [33]. A normalized score for a certain impact category is obtained by determining the ratio of the category indicator result of the product and that of a reference system [24]. Weighting represents the relative importance of each impact and calculated by multiplying all normalized impact categories with each weighting factors [33].

There is no standardized list of impact categories [30]. Guinée et al. [20] has tabulated most of the impact categories in the 'Handbook of LCA'. Wu et al. [68] in a LCA of maize stover bioethanol considered global warming, carbon monoxide (CO), volatile organic compounds (VOCs), nitrogen oxide (NO_x), sulfur oxide (SO_x) and particulate matter with diameters smaller than 10 micrometers (PM_{10}). Monti et al. [41] in their LCA on energy crops fractionated human and environmental toxicity into various classes (i.e. human, fresh water, marine and terrestrial toxicity) and they did not include photochemical oxidation.

Most of the LCIA practices use European database to establish the characterization, normalization and weighting value, however, using European database for LCA practice outside the Europe might not be able to reflect the actual environmental scenario of that region. Therefore, it is essential to create a local database of normalization and weighing values by using the pollution data collected from the local area. This will provide a more representative value of pollutant loads when applied in LCA studies of that area and enhance the accuracy of the results obtained in the studies.

4.8 Allocation

Allocation is the process of assigning to each of the functions of a multiple-function system only, those environmental burdens associated with that function [3]. Reap et al. [52] defines allocation as a procedure of appropriately allocating the environmental burdens of a multi-functional process among its functions or products. Allocation step is one of the determining steps that how much of the environmental burdens caused by the multi-functional process should be apportioned to each product or function. The inappropriate allocations could lead to incorrect LCA results. Ekvall and Tillman [11] mention eight allocation procedures that they considered fair or reasonable based on the procedures underlying perspectives. Curran [8] reviewed allocation approaches and concluded that no single method provides a general solution.

ISO [31, 33] recommends, avoid allocation when possible either by dividing the unit processes into sub-processes (substitution approach) and gathering the

required environmental burden data and/or by expanding the product system boundaries to include additional functions related to the co-products. The substitution approach can be a fundamentally sound method which increases the scope of LCA; it is not generally used if the main process does not include for co-products, by-products or residues. If allocation cannot be avoided, allocate the environmental burdens of each product based on their underlying physical relationships (e.g. carbon, energy, mass, volume, etc.). If allocation based on physical relationships cannot be done, allocate the environmental burdens of each product based on other relationships (e.g. economic value). However, this method is not useful because prices are determined for a number of market factors that are not related to the energy content [63].

Kim and Dale [35] in their study of ethanol production from various feedstocks (i.e. dry and wet milling, soybean oil, soybean meal from soybean milling) adopted an expanded system approach. They concluded that this approach could be used to compare the environmental burdens associated with ethanol to those associated with fossil fuel. However, this approach would be over elaborate for an LCA study in which the goal is to compare the environmental burdens between different ethanol production technologies. Gnansounou et al. [19] stated that the net GHG emissions of ethanol production may vary with allocation method adopted (mass, energy or carbon content or economy), with carbon content being the most favorable and economy being the least favorable.

The choice of the allocation methodology is essential for the environmental impact assessment. Since environmental benefits are the main reasons for considering biofuels, it is important to grasp this and give special attention on allocation in LCA studies of biofuels.

4.9 Interpretation

Interpretation is the step in which the results of the analysis and all choices and assumptions made during the course of the analysis are evaluated in terms of soundness and robustness, and overall conclusions are drawn. The main elements of the interpretation phase are an evaluation and analysis of results and the formulation of the conclusions and recommendations of the study [20]. ISO 14043 (2000E) defines Interpretation as a systematic procedure to identify, qualify, check and evaluate information from the results of the LCI and/or LCIA of a product system, and present them in order to meet the requirements of the application as described in the goal and scope of the study. It can be regarded as containing procedural steps (e.g. completeness check) as well as numerical steps (e.g. sensitivity check). The procedural approaches include all types of analyses that deal with the data and results in relation to other sources of information, like expert judgements, reports on similar products, intuition, reputation of data suppliers, etc. The numerical approaches explore the data in different ways, to produce different types of ‘smart’

data reduction that provides an indication of reliability, key issues, discernibility, robustness, etc. [25].

Reap et al. [53] concluded that in interpretation, the aggregation is the dominating problem and weighting or valuation of inventory or impact data is required to produce a single figure of merit. Unfortunately, weighting and valuation introduce subjectivity, subjectivity that is not always satisfactorily handled by current methods in decision science. Inaccuracies and variability inherent in modeled systems result in a high degree of aggregated uncertainty before reaching to the interpretation step and making good decisions under this potentially severe level of uncertainty is challenging.

5 Conclusions

LCA is a tool to define the environmental burdens from a product, process or activity by identifying and quantifying energy and materials usage, as well as waste discharges, assessing the impacts of these wastes on the environment and it also evaluates the opportunities for environmental improvements over the whole life cycle. The goal, scope and system boundaries should be defined well and a functional unit should be expressed in an output based on energy associated with energy systems. Well to wheel system is recommended for the assessment of biofuels production system. Allocation by substitution or expended system or on the basis of carbon content is recommended. Thus, an LCA of biofuels will help in accessing their sustainability and adopting the policies for their promotions.

References

1. Agrawal AK (2007) Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. Prog Energy Combust Sci 33:233–271
2. Aleklett K, Campbell CJ (2003) The peak and decline of world oil and gas production. Miner Energy 18:35–42
3. Azapagic A, Clift R (1999) Allocation of environmental burdens in co-product systems: product-related burdens. Int J Life Cycle Assess 4:357–369
4. Biswas WK, Barton L, Carter D (2011) Biodiesel production in a semiarid environment: a life cycle assessment approach. Environ Sci Technol 45:3069–3074
5. Brennan L, Owende P (2010) Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev 14:557–577
6. Brent AC, Rohwer MB, Friedrich E, Von Blottnitz H (2002) Status of life cycle assessment and engineering research in South Africa. Int J Life Cycle Assess 7:167–172
7. Carere CR, Sparling R, Cicek N, Levin DB (2008) Third generation biofuels via direct cellulose fermentation. Int J Mol Sci 9:1342–1360
8. Curran MA (2007) Co-product and input allocation approaches for creating life cycle inventory data: a literature review. Int J Life Cycle Assess 1:65–78

9. Davis SC, Anderson-Teixeira KJ, DeLucia EH (2009) Life-cycle analysis and the ecology of biofuels. Trends Plant Sci 14:140–146
10. de Eicker MO, Hischier R, Kulay LA, Lehmann M, Zah R, Hurni H (2010) The applicability of non-local LCI data for LCA. Environ Impact Assess Rev 30:192–199
11. Ekvall T, Tillman A-M (1997) Open-loop recycling: criteria for allocation procedures. Int J Life Cycle Assess 2:155–162
12. Ekvall T, Weidema BP (2004) System boundaries and input data in consequential life cycle inventory analysis. Int J Life Cycle Assess 9(3):161–171
13. Elkington J (1998) Cannibals with forks—the triple bottom line of 21st century business. New Society, Canada
14. Elsayed MA, Matthews R, Mortimer ND (2003) Carbon and energy balances for a range of biofuels options. Final report—prepared for the department of trade and industry renewable energy programme. Unit of Sheffield Hallam University and Forest Research, UK
15. Farrell AE, Pelvin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM (2006) Ethanol can contribute to energy and environmental goals. Science 311:506–508
16. Fulton L, Howes T, Hardy J (2004) Biofuels for transport: an international perspective. International Energy Agency (IEA), Paris
17. Gabrielle B, Gagnaire N (2008) Life-cycle assessment of straw use in bio-ethanol production: a case study based on biophysical modeling. Biomass Bioenergy 32:431–441
18. Gnansounou E (2011) Assessing the sustainability of biofuels: a logic-based model. Energy 36:2089–2096
19. Gnansounou E, Dauriat A, Villegas J, Panichelli L (2009) Life cycle assessment of biofuels: energy and greenhouse gas balances. Bioresour Technol 100:4919–4930
20. Guinée JB, Gorrée M, Heijungs R, Huppes G, Kleijn R, de Koning A, van Oers L, Wegener Sleeswijk A, Suh S, Udo de Haes HA, de Bruijn H, van Duin R, Huijbregts MAJ (2002) Handbook on life cycle assessment: operational guide to the ISO standards. Kluwer Academic, USA
21. Gullison RE, Frumhoff PC, Canadell JG, Field CB, Nepstad DC, Hayhoe K, Avissar R, Curran LM, Friedlingstein P, Jones CD, Nobre C (2007) Tropical forests and climate policy. Science 316:985–986
22. Haye S, Hardtke CS (2009) The roundtable on sustainable biofuels: plant scientist input needed. Trends Plant Sci 14(8):409–412
23. He Y, Wang S, Lai KK (2010) Global economic activity and crude oil prices: a cointegration analysis. Energy Econ 32:868–876
24. Heijungs R, Guinée J, Kleijn R, Rovers V (2007) Bias in normalization: causes, consequences, detection and remedies. Int J LCA 12:211–216
25. Heijungs R, Kleijn R (2001) Numerical approaches towards life cycle interpretation five examples. Int J LCA 6:141–148
26. IEA (2007) World energy outlook 2007. International Energy Agency, Paris
27. IEA (2009) IEA scoreboard 2009–35 key energy trends over 35 years. International Energy Agency, France
28. IEA (2010) CO_2 emissions from fuel combustion highlights (2010 edn). International Energy Agency, France
29. IEA (2011) Technology roadmap biofuels for transport. International Energy Agency, France
30. IFEU (2000) Bioenergy for Europe: which ones fit best?–a comparative analysis for the community. Institute for Energy and Environmental Research, Heidelberg
31. ISO (1998) ISO 14041: environmental management—life cycle assessment—goal and scope definition and inventory analysis. ISO 14041:1998(E), International Standards Organization
32. ISO (2005) 14040-Environmental management—life cycle assessment—requirements and guidelines. International Standard Organisation p 54
33. ISO (2006) ISO 14044: environmental management—life cycle assessment—requirements and guidelines. ISO 14044:2006(E), International Standards Organization

34. Jensen AA, Hoffman L, Møller BT, Schmidt A, Christiansen K, Elkington J, van Dijk F (1997) Life-cycle assessment (LCA)—a guide to approaches, experiences and information sources. Environmental issues series no. 6. European Environment Agency, Copenhagen
35. Kim S, Dale DE (2002) Allocation procedure in ethanol production system from corn grain: I. System expansion. Int J Life Cycle Assess 7:237–243
36. Kiwjaroun C, Tubtimdee C, Piumsomboon P (2009) LCA studies comparing biodiesel synthesized by conventional and supercritical methanol methods. J Clean Prod 17:143–153
37. Korres NE, Singh A, Nizami AS, Murphy JD (2010) Is grass biomethane a sustainable transport biofuel. Biofuels Bioprod Biorefin 4:310–325
38. Korres NE, Thamsiriroj T, Smyth BM, Nizami AS, Singh A, Murphy JD (2011) Grass biomethane for agriculture and energy. In: Lichtouse E (ed) Genetics, biofuels and local farming systems, sustainable agriculture reviews 7. Springer, New York
39. Larson ED (2006) A review of life-cycle analysis studies on liquid biofuel systems for the transport sector. Energy Sustain Develop 10:109–126
40. Markevičius A, Katinas V, Perednis E, Tamasauskiene M (2010) Trends and sustainability criteria of the production and use of liquid biofuels. Renew Sustain Energy Rev 14:3226–3231
41. Monti A, Fazio S, Venturi G (2009) Cradle-to-farm gate life cycle assessment in perennial energy crops. Eur J Agron 31:77–84
42. Nigam PS, Singh A (2011) Production of liquid biofuels from renewable resources. Prog Energ Combust Sci 37:52–68
43. Onn CC, Yusoff S (2010) The formulation of life cycle impact assessment framework for Malaysia using eco-indicator. Int J Life Cycle Assess 15:985–993
44. Pant D, Singh A, Bogaert GV, Diels L, Vanbroekhoven K (2011) An introduction to the life cycle assessment (LCA) of bioelectrochemical systems (BES) for sustainable energy and product generation: relevance and key aspects. Renew Sustain Energy Rev 15:1305–1313
45. Pant D, Van Bogaert G, Diels L, Vanbroekhoven K (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 101:1533–1543
46. Phalan B (2009) The social and environmental impacts of biofuels in Asia: an overview. Appl Energy 86:S21–S29
47. Phetteplace HW, Johnson DE, Seidi AF (2001) Greenhouse gas emissions from simulated beef and dairy livestock systems in the United States. Nutr Cycl Agroecosyst 60:99–102
48. Pickett J, Anderson D, Bowles D, Bridgwater T, Jarvis P, Mortimer N, Poliakoff M, Woods J (2008) Sustainable biofuels: prospects and challenges. The Royal Society, London
49. Power N, Murphy JD (2009) Which is the preferable transport fuel on a greenhouse gas basis: biomethane or ethanol? Biomass Bioenergy 33:1403–1412
50. Prasad S, Singh A, Jain N, Joshi HC (2007) Ethanol production from sweet sorghum syrup for utilization as automotive fuel in India. Energy Fuels 21:2415–2420
51. Prasad S, Singh A, Joshi HC (2007) Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour Conserv Recycl 50:1–39
52. Reap J, Roman F, Duncan S, Bras B (2008) A survey of unresolved problems in life cycle assessment part 1: goal and scope and inventory analysis. Int J Life Cycle Assess 13:290–300
53. Reap J, Roman F, Duncan S, Bras B (2008) A survey of unresolved problems in life cycle assessment part 2: impact assessment and interpretation. Int J Life Cycle Assess 13:374–388
54. Reinhard J, Zah R (2011) Consequential life cycle assessment of the environmental impacts of an increased rapemethylester (RME) production in Switzerland. Biomass Bioenergy 35:2361–2373. doi:10.1016/j.biombioe.2010.12.011
55. SAIC (2006) Life cycle assessment: principles and practice. Scientific applications international corporation (SAIC), report no. EPA/600/R-06/060. National Risk Management Research Laboratory, Office of Research and Development, US Environmental Protection Agency, Cincinnati, Ohio

56. Schenk P, Thomas-Hall S, Stephens E, Marx U, Mussgnug J, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenerg Res 1:20–43
57. Silva Lora EE, Escobar Palacio JC, Rocha MH, Grillo Renó ML, Venturini OJ, del Olmo OA (2011) Issues to consider, existing tools and constraints in biofuels sustainability assessments. Energy 36:2097–2110
58. Singh A, Nigam PS, Murphy JD (2011) Renewable fuels from algae: an answer to debatable land based fuels. Bioresour Technol 102:10–16
59. Singh A, Nigam PS, Murphy JD (2011) Mechanism and challenges in commercialisation of algal biofuels. Bioresour Technol 102:26–34
60. Singh A, Olsen SI (2011) Algal biofuels: key issues, sustainability and life cycle assessment. In: Petersen LS, Larsen H (eds) Energy systems and technologies for the coming century. Proceedings, Risø international energy conference, 10–12 May 2011, pp 275–282
61. Singh A, Olsen SI (2011) Critical analysis of biochemical conversion, sustainability and life cycle assessment of algal biofuels. Appl Energy 88:3548–3555
62. Singh A, Olsen SI, Nigam PS (2011) A viable technology to generate third generation biofuel. J Chem Tech Biotech. doi:10.1002/jctb.2666 [Epub ahead of print]
63. Singh A, Pant D, Korres NE, Nizami AS, Prasad S, Murphy JD (2010) Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: challenges and perspectives. Bioresour Technol 101(13):5003–5012
64. Singh A, Smyth BM, Murphy JD (2010) A biofuel strategy for Ireland with an emphasis on production of biomethane and minimization of land-take. Renew Sustain Energy Rev 14(1):277–288
65. Spatari S, Zhang Y, MacLean HL (2005) Life cycle assessment of switchgrass- and corn stover-derived ethanol-fueled automobiles. Environ Sci Technol 39:9750–9758
66. UNCED (1992) Promoting sustainable human settlement development. Report of the United Nation conference on environment and development, vol 1, chap 7. United Nations Division for Sustainable Development, Rio de Janeiro
67. Wenzel H, Hauschild M, Alting L (1997) Environmental Assessment of Products. Vol. 1 Methodology, tools and case studies in product development. Kluwer Academic, USA
68. Wu M, Wang M, Huo H (2006) Fuel-cycle assessment of selected bioethanol production pathways in the United States. Argonne National Laboratory, Argonne ANL/ESD/06-7
69. Yan J, Lin T (2009) Bio-fuels in Asia. Appl Energy 86:S1–S10
70. Yang J, Xu M, Zhang X, Hu Q, Sommerfeld M, Chen Y (2011) Life-cycle analysis on biodiesel production from microalgae: water footprint and nutrients balance. Bioresour Technol 102:159–165
71. Zah R, Faist M, Reinhard J, Birchmeier D (2009) Standardized and simplified life-cycle assessment (LCA) as a driver for more sustainable biofuels. J Clean Prod 17:S102–S105
72. Zah R, Hischier R, Gauch M, Lehmann M, Boni H, Wager P (2007) Life cycle assessment of energy products: environmental impact assessment of biofuels. Bundesamt fur Energie, Bundesamt fur Umwelt, Bundesamt fur Landwirtschaft, Bern, p 20
73. Zhao R, Bean SR, Wang D, Park SH, Schober TJ, Wilson JD (2009) Small-scale mashing procedure for predicting ethanol yield of sorghum grain. J Cereal Sci 49(2):230–238