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POSGRADO

Posibilidades y retos de la bioenergía: Estado del arte y perspectivas futuras

Tesis que como Requisito para obtener el Grado de Maestro en Ciencias en Energías Renovables presenta:

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La amistad sólo podía tener lugar a través del desarrollo del respeto mutuo y dentro de un espíritu de sinceridad
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Índice

Resumen

La bioenergía es una agregación muy heterogéneo de diferentes materias primas, tecnologías de conversión y usos finales, que a menudo se promueve como una alternativa "verde" a los combustibles fósiles, mientras que los métodos biotecnológicos para mejorar el rendimiento de los biocombustibles han sido bien estudiados, especialmente en los cultivos y microorganismos. El objetivo de esta investigación es conocer el efecto potencial de la producción de bioenergía a gran escala sobre los indicadores sociales, económicos y del medio ambiente. Una búsqueda de la literatura se realizó utilizando la base de datos de ISI web of Knowledge, seleccionando sólo los trabajos publicados durante los últimos cinco años. Los tópicos elegidos fueron “bioenergy”, “review”, “biotechnology” y “biofuels”. Se estima que los bioenergéticos podrían cubrir las futuras carencias de combustibles fósiles, disminuir las emisiones de CO_2 y el calentamiento global. Sin embargo, el éxito de los bioenergéticos depende de la cantidad y calidad de la biomasa disponible y la capacidad de utilizarla de manera rentable para la producción de energía. Se necesitan avances biotecnológicos para desarrollar cultivos bioenergéticos más adaptados a las condiciones ambientales adversas, con una mayor tasa de crecimiento y alto valor calórico. La biotecnología deberá diversificar la agricultura, la silvicultura y la bioenergía y mejorar la calidad de vida de los agricultores y las empresas biotecnológicas deberán ser proveedores confiables de materias primas para la industria de los biocombustibles. El análisis del ciclo de vida y la evaluación de los daños colaterales son importantes para la evaluación de las ventajas y desventajas sociales y ambientales de la bioenergía. Se requiere una tecnología integrada y global para determinar los posibles efectos de los biocombustibles producidos, con el fin de desarrollar nuevas biotecnologías para dar forma a una transición hacia una civilización verdaderamente sustentable.

Abstract

Bioenergy is a very heterogeneous aggregation of different feedstocks, conversion technologies and end-uses, and are often promoted as a "green" alternative to fossil fuels, while the biotechnological approaches to improve the yield of biofuels have been well studied, especially in crops and microorganisms. The objective of this research is to know the potential effect of bioenergies production at large-scale on the social, economic and environment indicators. A literature search was undertaken using the ISI web of knowledge database, while we selected only papers published during the last five years. The topic selected were “bioenergy”, “review”, “biotechnology” and “biofuels”. Bioenergy production systems are sometimes claimed to be able to fill the future fossil fuel shortages as well as to decrease carbon dioxide emissions and global warming. However, the success of bioenergy production depends on the quantity and quality of biomass available and the ability to cost effectively utilize it for energy production. The global effects of the development of biofuel production will depend crucially on where and how feedstocks are produced. Advances in biotechnology are needed to develop bioenergy crops more adapted to adverse environmental conditions with higher growth rate and high caloric value. Biotechnology will lead to new opportunities to diversify agriculture, forestry and bioenergy and improve the quality of life of farmers, so biotech companies play a key role as reliable suppliers of raw materials for the biofuels industry. Life-cycle analysis and evaluation of external costs are important for assessing the social, economic, and environmental advantages and disadvantages of bioenergy system. An integrated and holistic technology is required to determine the potential effects of produced biofuels, in order to develop new biotechnologies to shape a real transition to a truly sustainable civilization.

I. Introducción

En todo el mundo, durante los últimos años se ha incrementado el interés por buscar alternativas energéticas que contribuyan a mitigar el impacto negativo al ambiente. En México se están apoyando a las Instituciones de Educación Superior para que investiguen la forma de aprovechar al máximo los recursos naturales de que se dispone, para que éstos se utilicen como una fuente alterna de energía que contribuya al desarrollo sustentable y al bienestar social. En la actualidad, son varios los problemas que el mundo moderno está enfrentando, entre ellos el Cambio Climático, la emisión de gases de efecto invernadero (dióxido de carbono [CO_2], metano [CH_4] y óxido nitroso [N_2O], principalmente), la contaminación y la dependencia energética hacia los combustibles tradicionales (carbón, petróleo y gas natural). Se estima que de seguir el ritmo de consumo similar al actual, las reservas de combustibles fósiles se agotarán en un plazo de entre 50 y 100 años; por el contrario los recursos naturales renovables pueden regenerarse y estar disponibles en cantidades relativamente constantes.

En la era contemporánea la necesidad de disponer de fuentes de energía renovable se ha convertido en algo imprescindible para el ser humano. El potencial energético eólico, solar (térmica y fotovoltaica), hidráulico, geotérmico y la biomasa (también llamada bioenergía), son fuentes de obtención de energías sin destrucción del ambiente, además renovables, que han sido investigadas y desarrolladas con excelentes resultados en las últimas décadas.

De igual manera debemos tener en cuenta que el desarrollo de la sociedad humana en el mundo está basado en el consumo de grandes cantidades de energía proviene en su mayoría, de los combustibles fósiles, los cuales se están agotando. Alrededor de 1.5 millones de personas en todo el mundo todavía no tienen acceso a la electricidad, y aproximadamente 2600 millones dependen de la madera, paja, carbón o estiércol para cocinar sus alimentos diarios (REN21, 2010).

Las tecnologías modernas de la bioenergía (es la biomasa que puede ser generada directamente, procesada en combustibles sólidos secos y densificados, o bien convertida en combustibles líquidos o gaseosos, utilizando las denominadas tecnologías de primera o segunda generación, dependiendo del nivel de desarrollo) que producen combustibles para calefacción, electricidad y transporte están avanzando rápidamente, con mucho del reciente interés centrado en los biocombustibles líquidos, en particular el etanol y el biodiesel. Actualmente, Estados Unidos y Brasil dominan la industria de combustibles líquidos, pero hoy en día muchos otros gobiernos están considerando activamente el papel adecuado de los biocombustibles en sus futuras agendas energética (ONU-Energía, 2007).

El rápido desarrollo mundial de la bioenergía moderna presenta claramente un amplio rango de oportunidades, pero también acarrea muchas desventajas y riesgos. La experiencia en cuanto a los impactos sociales, económicos y ambientales asociados es limitada, y los tipos de impacto dependerán ampliamente de las condiciones locales y de los marcos de trabajo de las políticas implementadas para apoyar el desarrollo de la bioenergía (ONU-Energía, 2007). El desarrollo de nuevas industrias de bioenergía podrá proporcionar servicios de energía limpia a millones de personas que actualmente carecen de ella y al mismo tiempo, generar ingresos y crear empleos en las zonas más pobres del mundo. El rápido crecimiento en la producción de biocombustibles líquidos de primera generación obtenidos a partir del azúcar, almidón, aceite vegetal o grasas animales (los cuales utilizan tecnología convencional), elevarán los precios de la materia prima agrícola y podría tener efectos negativos a nivel económico y social, particularmente entre los más necesitados que gastan una gran parte de sus ingresos en alimentos.

Con base en literatura publicada, consideramos que el proyecto de investigación aquí planteado tiene impacto socioeconómico, científico y tecnológico. El impacto socioeconómico está relacionado con el uso de los residuos para obtener ganancias a través de la generación de biocombustibles e incrementar la calidad de vida de las poblaciones. El impacto científico se alcanza considerando que con este análisis se podrán hacer investigaciones sobre el aprovechamiento de la biomasa que se genera en el mundo, principalmente en México y tanto los investigadores como los estudiantes de diferentes disciplinas (Energías Renovables, Biotecnología, Química, etc.), considerarán aspectos sociales, económicos, ambientales y biotecnológicos antes de hacer propuestas de aprovechamiento para la generación de biogás, bioetanol, biodiesel o electricidad, entre otros. El impacto tecnológico se consigue debido a que la investigación científica vinculada con este proyecto permitirá hacer nuevas propuestas o innovaciones a las tecnologías ya existentes.

El objetivo de esta investigación es conocer el efecto potencial que podría tener la generación y uso de bioenergías a gran escala sobre indicadores sociales, económicos y ambientales. Los objetivos particulares planteados son: i) determinar las principales fuentes de biomasa; ii) definir los indicadores sociales, económicos y ambientales más relevantes, ligados a los bioenergéticos y iii) determinar los retos tecnológicos que implica el uso de bioenergéticos a gran escala. El alcance de la investigación es publicar al menos un artículo científico de revisión en una revista científica internacional con arbitraje estricto.

La hipótesis planteada es: A nivel mundial se ha impulsado el uso de las bioenergías tomando en cuenta de manera aislada su impacto social, económico y ambiental, por lo que es necesario considerar enfoques holísticos que nos permitan lograr la transición hacia un desarrollo sustentable.

El Estado del Arte presenta los aspectos principales de los dos artículos que contiene esta tesis (Uno publicado y otro en versión R1), por lo que, con el objeto de evitar reiterar la información el Estado del Arte es breve.

I.1 Estado del Arte

La energía renovable es la energía generada a partir de recursos naturales como, por ejemplo, agua, luz solar, viento, lluvia, mareas, olas, fuentes geotérmicas y fuentes de biomasa como microorganismos, plantas, estiércoles, biosólidos y residuos orgánicos de origen doméstico. En todo el mundo hay un creciente interés en el uso de biocombustibles sólidos, líquidos y gaseosos, debido principalmente a: i) beneficios políticos y de soberanía nacional (algunos países podrían reducir substancialmente la dependencia a la importación de energéticos tradicionales), ii) la creación de empleaos (los biocombustibles a partir de biomasa crean alrededor de 20 veces más empleos que los combustible tradicionales y iii) los beneficios ambientales como la mitigación de la emisión de gases efecto invernadero, la reducción de lluvia ácida y el mejoramiento de la calidad de los suelos (Van Loo and Koppejan, 2008).

En Estados Unidos de América y muchos otros países, los combustibles fósiles proveen alrededor del 85% de sus necesidades energéticas (Scheller et al., 2010), lo cual no es sustentable por dos razones: i) las reservas de combustibles tradicionales son limitadas y ii) el uso constante de energéticos tradicionales incrementa las emisiones de CO_2. Alrededor de 1500 millones de personas en todo el mundo no tiene acceso a la electricidad y 2600 millones de personas depende de la madera, paja, carbón y estiércol seco para cocinar sus alimentos (REN21, 2010), lo cual demuestra que es necesaria un fuente de energía barata y ambientalmente amigable. Las bioenergías han ganado fuerte aceptación por políticos, científicos, ambientalistas, empresarios agrícolas y público en general. Ellos consideran que las bioenergías contribuyen a la solución de muchos problemas como el efecto invernadero, los crecientes precios del petróleo, la dependencia energética y el rezago rural (Russi, 2008). Sin embargo, un cuidadoso análisis de literatura sobre la bioenergía revela que no hay consenso sobre el potencial de biomasa entre los investigadores de todo el mundo pero además, es claro que sus evaluaciones difieren fuertemente.

Algunos de los cuellos de botella más críticos para el incremento del uso de biomasa para la producción de energía son el costo de las operaciones logísticas, la competencia con los alimentos y el cambio de uso de suelo. Por lo anterior, investigación adicional es necesaria para desarrollar mejores métodos para producir bioenergía, así como mejores procedimientos de evaluar y verificar el desempeño ambiental de los biocombustibles.

Gases efecto invernadero y producción de bioenergéticos

La producción de bioenergía está estrechamente ligada con la emisión de gases efecto invernadero a la atmósfera, por lo que es importante considerar fuentes de biomasa a base de CO_2 neutral (Cebrucean et al., 2010). Para algunos autores la biomasa es uno de las pocas opciones para remplazar los energéticos tradicionales (McKendry, 2002; Munir et al., 2009), a pesar de que se ha reportado que el cultivo intensivo, el uso de abonos orgánicos y fertilizantes químicos incrementan las concentraciones de CO_2, N_2O y CH_4 en la atmósfera (Lopez-Valdez et al., 2011; Fernandez-Luqueno et al., 2010). Adicionalmente se ha reportado que la generación de electricidad a partir de cultivos libera cero emisiones de gases efecto invernadero y fija 17,981 toneladas de CO_2-C equivalentes año^{-1} (Carneiro and Ferreira, 2012). Sin embargo, si las emisiones de CO_2 son contadas durante todo el sistema de producción agrícola, sí se detectará una emisión neta de CO_2 i.e. después de hacer una evaluación integral de la sustentabilidad del sistema agrícola, la bioenergía podría obtenerse a expensas de emisiones de gases efecto invernadero a la atmósfera y de la salud ambiental, por lo que es necesario un esfuerzo sustancial para obtener bioenergéticos sin emisiones de CO_2-C equivalentes para estimular la producción de estos a gran escala.

La huella hídrica de la bioenergía

La promoción del uso de bioenergéticos para reducir los gases efecto invernadero ha incrementado el uso de agua, especialmente durante el cultivo de biomasa (Gheewala et al., 2011). Si bien las necesidades de agua de los cultivos están en función del tipo de cultivo, el sistema de producción agrícola y las condiciones climáticas (Abdullah 2010), es importante reconocer que intensificar los sistemas de producción agrícola e incrementar las superficies para ese uso demandará mayor cantidad de agua. Es decir, incrementar a gran escala el cultivo de plantas con potencial bioenergético crea tanto oportunidades como desafíos para el sector hídrico. Cultivos como maíz, caña de azúcar o palma de aceite requieren grandes cantidades de agua y si esta es escasa los rendimientos serán bajos (Fraiture et al., 2008). Es importante considerar que en el futuro será relevante e indispensable evaluar el impacto de las bioenergías sobre el recurso agua porque alrededor del 80% de la población mundial está en riesgo ligado a la calidad y cantidad de agua disponible (Vorosmarty et al., 2010) mientras que además, la rápida expansión de los cultivos con potencial bioenergético pueden afectar patrones hidrológicos regionales (Subhadra 2010). Los estudios futuros sobre bioenergía tendrán que tener en cuenta el factor agua, de modo que el equilibrio entre la mitigación del cambio climático, la oferta de bioenergéticos y la escasez de agua debe ser abordado.

Bioenergía y sustentabilidad: un desafío creciente

Se publica que la bioenergía podría substituir y por tanto disminuir el impacto de los combustibles fósiles. Sin embargo, el crecimiento significativo de los recursos bioenergéticos podría presentar retos a la sustentabilidad y poner en riesgo los componentes de rendimiento de los cultivos bioenergéticos. Además, la producción a gran escala de esos cultivos podría competir por suelo y agua con los cultivos de importancia nutricional y el cambio de uso de suelo podría implicar impactos ambientales y sociales (Powers et al., 2011). Si el cultivo de plantas con potencial energético se incrementa, implicará un incremento significativo en el uso de pesticidas y fertilizantes (Lavigne and Powers, 2007), lo cual conllevará a incrementar la contaminación ambiental, aumentarán las lixiviaciones y volatilizaciones de nitrógeno, la pérdida de C del suelo y la pérdida de suelo por erosión (Karp and Shield, 2008). Por lo anterior, para alcanzar un suministro sustentable y constante de biomasa para la producción de bioenergéticos se deberán incluir aspectos de rendimiento total, calidad de agua, calidad de suelo, contaminación, emisión de gases efecto invernadero, balance neto de energía y productividad del cultivo, entre otros parámetros (Blengini et al., 2011).

Biotecnología y bioenergía: desarrollo conjunto para generar oportunidades

Las aplicaciones biotecnológicas juegan un papel fundamental en la solución de problemas relacionados con el sector industrial, alimenticio y energético. Mejoras en la composición y estructura bioquímica de cultivos energéticos incrementará la producción de energía por tonelada de biomasa, mejorará su valor calórico y su efecto sobre la emisión de gases efecto invernadero y calentamiento global (Abdullah, 2010). La conversión de biomasa lignocelulolitica a azúcares fermentables, representa uno de los principales retos para utilizar recursos renovables en lugar de combustibles fósiles, para cubrir la demanda de energía (Lynd et al., 2008; Miller and Keller 2009; Moon et al., 2010). Los híbridos de maíz actuales y de otros muchos cultivos, fueron mejorados durante miles de años por cruzas tradicionales, luego se puso en práctica la mejora genética a través de técnicas modernas y más recientemente, la mejora de plantas se realiza mediante herramientas biotecnológicas (Heaton et al., 2008). En los últimos años avances genéticos y biotecnológicos han permitido generar y cultivar arboles maderables para usarlos como fuente de bioenergía (Seguin, 2011), lo cual implica una ventaja pues contribuye a la fijación de C y a la preservación de los bosques. Una avance de gran relevancia es la generación y uso de plantas transgénicas, sin embargo deberá ser necesario considerar aspectos regulatorios y los riesgos de que esas plantas se liberen en el ambiente. Del mismo modo, la pérdida de genes de los cultivos genéticamente modificados y la posible transferencia de genes a otros cultivos también debe ser investigada. Al respecto, una estrategia denominada mitigación transgénica podría ser efectiva en los cultivos transgénicos de interés bioenergético (DiFazio et al., 2012; Moon et al., 2010). Un gen primario de interés podría tener un gene mitigante el cual sea positivo o neutral para cultivos transgénicos pero negativo o deletéreo para cultivos hospederos potenciales no transgénicos (Al-Ahmad et al., 2004). Es importante no olvidar que la mejor manera de controlar las dispersión de genes es a través de prácticas de manejo (DiFazio et al., 2012). Existen avances tecnológicos muy relevantes pero aún hay varios desafíos importantes que deben ser abordados con éxito, con el fin de estimular la producción de biomasa sin introducir riesgos ambientales, sociales o económicos, es decir, debemos lograr producir suficiente biomasa sin incurrir en graves daños al medio ambiente y al sistema de suministro de alimentos.

Costo ambiental, económico y energético de la bioenergía

La organización mundial para la alimentación estima que la producción de alimentos se deberá incrementar 70% en un periodo entre el 2005 y 2050 (FAO, 2009). Sin embargo, el surgimiento y desarrollo de los biocombustibles tiene el potencial de modificar las tendencias proyectadas y causar que la demanda se incremente (FAO, 2009), mientras que se estima que la demanda global de combustibles para medios de transporte se podría incrementar incluso más rápido (USDE, 2006), por lo que es de suma importancia que los biocombustibles no compitan con el suministro de alimentos. Adicionalmente, la producción de bioenergía incrementa la presión sobre el recurso agua y suelo, por lo que la productividad de alimentos y materia prima vegetal deberá ser mejorada porque además, los biocombustibles para transporte son el sector que más rápido está creciendo (IEA-Bioenergy, 2009). Ahlgren et al. (2010) investigó el uso de suelo, el impacto ambiental y el uso de energía fósil cuando usaron biogás en lugar de gas natural en la producción de fertilizante nitrogenado. El biogás fue producido por digestión anaerobia de pastos y maíz, sus cálculos muestran que una hectárea de suelo agrícolas puede producir 1.7 toneladas métricas por año de N en forma de nitrato de amonio o 3.6 toneladas métricas de maíz y el impacto sobre el calentamiento global fue más bajo cuando se produjo fertilizante nitrogenado de biomasa comparado con el gas natural. En el escenario de biomasa solo de 2 a 4 MJ/Kg N de energía fósil fue necesaria, mientras que 35 MJ/Kg N fue requerida cuando se utilizó gas natural. No obstante, los biocombustibles son ampliamente vistos como sustitutos de los combustibles fósiles para compensar la inminente caída de la producción petrolera y para mitigar el aumento emergente en las emisiones de gases de efecto invernadero. Este punto de vista es, sin embargo, sobre la base de un análisis demasiado simple, centrándose en una sola pieza en el mosaico entero del complejo tecno-sistema de los biocombustibles y tales enfoques parciales pueden conducir fácilmente a la tendencia ideológica basada en la preferencia política. Una de las fortalezas más importantes de la biomasa es la promoción del desarrollo de las zonas rurales, reducir el éxodo rural y el fortalecimiento de la industria local. Otro aspecto muy importante es la posibilidad de crear puestos de trabajo principalmente en las regiones menos favorecidas de todo el mundo. Como debilidades potenciales se refiere con frecuencia al posible uso de la tierra que puede ser necesaria para la producción de alimentos (Carneiro y Ferreira, 2012). Además, es bien sabido que todavía hay también una falta de conocimiento sobre los cultivos energéticos. Por lo tanto, se necesitan políticas integradas de energía, uso de la tierra y el agua. La contribución de la bioenergía para satisfacer la demanda mundial de energía se puede ampliar de manera significativa en el futuro, proporcionando ahorros de gases de efecto invernadero y otros beneficios ambientales, así como contribuir a la seguridad energética, la mejora de la balanza comercial, proporcionando oportunidades para el desarrollo social y económico en las comunidades rurales, y la mejora de la gestión de recursos y residuos. Con el aumento de la población humana en todo el mundo, más tierra puede ser necesaria para producir alimentos para el consumo humano o animal, que es un desafío potencial de bioenergía, por lo que la bioenergía podría ser particularmente útil en situaciones específicas en las que los niveles de contaminación son importantes, por ejemplo en la minería, áreas protegidas, ambientes marinos costeros, etc. De acuerdo con los últimos informes, la bioenergía podría ser utilizada como un complemento a otras formas de energía, pero no como una fuente primaria. Además, en cada país, muchos temas diferentes se deben tomar en cuenta, no sólo la producción de energía o el costo económico, sino también los factores sociales y ambientales, antes de poner en marcha una política bioenergética. Por otra parte, los recursos de biomasa deben ser producidos con altos estándares ambientales, por lo que cada país en el mundo tiene que formular y poner en práctica una serie de políticas y programas innovadores, para promover las tecnologías de bioenergía antes de poner en marcha programas de energía renovable.

Biomasa: retos y oportunidades

Biomasa es el material orgánico no fosilizado y biodegradable originado de plantas, animales y microorganismos (Carneiro and Ferreira, 2012). La biomasa es una fuente de energía heterogénea que puede ser utilizada para satisfacer una variedad de necesidades de energía en las viviendas o industrias, incluyendo la generación de electricidad, la calefacción de casas y combustible de vehículos.

Hoy en día, la biomasa suministra alrededor del 50 EJ en el mundo, lo que representa el 10% del consumo anual de energía primaria mundial (IEA-Bioenergía, 2009). Un problema asociado con la producción de biomasa es el uso de suelo en el conflicto entre la producción de alimentos y la bioenergía. Muchos cultivos alimentarios tradicionales, como un maíz, azúcar y aceites vegetales también son algunas de las materias primas energéticas más utilizadas. Por otra parte, la producción de alimentos puede ser desplazada de las tierras agrícolas por la producción de cultivos energéticos, contribuyendo al aumento del precio de los alimentos. Otro problema serio asociado con la producción de biomasa son las emisiones de gases de efecto invernadero procedentes del manejo del suelo y el cambio de uso de suelo. Esto se refiere a las emisiones de gases de efecto invernadero (especialmente CO_2, CH_4 y N_2O) resultantes de los insumos agrícolas (como fertilizantes), prácticas de manejo y los cambios de uso del suelo (cuando los bosques, las praderas y otros ecosistemas son talados para producir cultivos) asociados a la producción de biomasa.

Es importante que los mercados de biomasa aporten un valor añadido a los productos de la biomasa, los residuos y las tierras productivas. Por lo tanto el desarrollo de la producción de biomasa también plantea estos desafíos, especialmente las emisiones agrícolas de gases de invernadero, el efecto del cambio de uso del suelo, los ecosistemas y el impacto asociado a la tala de los bosques y los efectos indirectos creados por los cambios en los mercados de materias primas de biomasa y de alimentos. Las posibilidades de producción de biomasa en el sector agrícola se han investigado extensamente por todo el mundo (Schindler, 2010; Jasiulewicz, 2010). Nijsen et al., (2012), hizo un primer intento global detallado, para estimar el potencial de la bioenergía en áreas degradadas. Dependiendo del tipo de cultivo, el potencial se estima en 190 EJ año^{-1} en todo el mundo, de los cuales alrededor de 25 a 32 EJ año^{-1} son en tierras no clasificadas actualmente como cultivo, pastizales o bosques. Áreas degradadas en todo el mundo pueden ser una promesa para la producción de bioenergía con poco impacto negativo en la producción de alimentos, la biodiversidad o las emisiones de gases de efecto invernadero. Aunque los energéticos renovables a partir de biomasa se consideran una de las fuentes con mayor potencial, para contribuir a las necesidades energéticas de las economías desarrolladas y en vías de desarrollo en todo el mundo, y teniendo en cuenta que los esfuerzos para que los biocombustibles a partir de recursos renovables aumenten en los últimos años, en la actualidad la producción de bioenergía a partir de diferentes materias primas derivadas de biomasa se siguen desarrollando. Sin embargo, es necesario tener en cuenta y afrontar los retos sociales, económicos, ambientales y tecnológicos relacionados con la producción de bioenergía a partir de biomasa.

Recientemente, diferentes investigaciones han revisado los cultivos modificados genéticamente con el fin de mejorar los cultivos de biocombustibles, por ejemplo, Vega-Sánchez y Ronald (2010), Arruda (2012), Calviño y Messing (2012), Parry y Hawkesford (2012). Es bien sabido que un número de recursos genómicos se ha desarrollado en varias especies de cultivos durante las últimas tres décadas y proporcionan una plataforma para el mejoramiento de cultivos comerciales. Sin embargo, para mejorar las características importantes de los cultivos bioenergéticos, al día de hoy no se dispone de varios recursos genómicos, tales como: mapas de ligamiento, las tecnologías de secuenciación de alto rendimiento, bases de datos de etiquetas de secuencia de expresión, secuencias del genoma, chips de ADN, etc. Hoy en día algunos científicos están trabajando para mejorar la asimilación de carbono fotosintético en plantas C3, C4 o CAM, utilizando la ingeniería genética (Cheng-Jiang et al., 2012; Weissmann y Brutnell, 2012; Work et al., 2012) y para incrementar los rendimientos y disminuir la concentración de CO_2 atmosférico. Hay que recordar que hay tres tipos de asimilación fotosintética de CO_2, y por lo tanto tres tipos de plantas: (i) las plantas C3, en las que el CO_2 atmosférico es asimilado directamente a través de la vía fotosintética C3, por ejemplo, el arroz (*Oryza sativa* L.), trigo (*Triticum aestivum* L.), soya (*Glycine max* L.), y patata (*Solanum tuberosum* L.), (ii) las plantas C4 evolucionaron la ruta fotosintética, por ejemplo, maíz (*Zea mays* L.) y caña de azúcar (*Saccharum officinarum* L.), y (iii) las plantas CAM (metabolismo ácido de las crasuláceas), en el que los estomas se cierran durante el día y abren sólo por la noche, cuando disminuye la temperatura y se eleva la humedad.

El nitrógeno es un nutriente importante que es determinante del rendimiento de cultivos y contribuye de manera fundamental a la calidad de las proteínas. Sin embargo, el nitrógeno es caro de producir, distribuir y aplicar, lo que resulta en una gran huella de carbono. Desde 1909 el proceso Haber-Bosch convierte el gas nitrógeno en amoníaco, que hoy en día ha permitido el crecimiento de los cultivos utilizados en la fabricación de biocombustibles. Los biocombustibles a base de plantas o a base de algas requieren la aplicación de fertilizante nitrogenado. El nitrógeno reducido es asimilado por la planta o las algas para hacer proteínas y ácidos nucleicos, los cuales no se utilizan para la producción de combustible. En cambio, el alto contenido de nitrógeno de los residuos se utiliza principalmente para la alimentación animal y finalmente, dan lugar a la dispersión de nitrógeno en el suelo. Se ha reportado que el reciclaje del amoníaco de los residuos ricos en proteínas, como un fertilizante para materia prima, podría cerrar el ciclo del nitrógeno (Huo et al., 2012). Además, las investigaciones para disminuir la escasez de N en el suelo para los cultivos de biocombustibles no se han desarrollado, de modo que es necesario realizar investigación con el fin de aumentar la asociación (simbiosis) entre las plantas y diazótrofos. Por lo que podría haber un aumento en los rendimientos de biomasa, es decir biocombustibles, sin emisión de gases de efecto invernadero adicional, ni contaminación generada por efecto de la fertilización.

Sistemas microbianos y algas para producción de biomasa

El cultivo de microalgas como materia prima alternativa para la producción de biocombustibles ha recibido mucha atención en los últimos años debido a su rápida tasa de crecimiento y la capacidad de acumular gran cantidad de lípidos e hidratos de carbono en el interior de sus células para la producción de biodiesel y bioetanol, respectivamente. Sistemas de microalgas tienen la ventaja de que pueden producir una amplia gama de materias primas para la producción de una serie de biocombustibles, incluyendo el biodiesel, bioetanol, biometano y biohidrógeno. Además, las microalgas tienen potencial en algunas áreas como la nutrición, la acuicultura, la medicina, el medio ambiente, los cosméticos y los productos farmacéuticos.

Las algas son un grupo muy diverso de organismos y no es sorprendente que diferentes especies de algas producen diferentes compuestos que podrían ser utilizados como materia prima para combustibles alternativos. Cinco componentes o productos de algas comúnmente estudiados y útiles para generar combustibles alternativos son: i) lípidos para la producción de biodiesel, ii) carbohidratos para producir etanol, hidrógeno o metano, iii) isoprenoides de la gasolina, iv) biomasa para combustión directa, digestión anaeróbica o conversión termoquímica, y v) síntesis directa de gas hidrógeno (Rosenberg et al., 2008; Srirangan, 2011).

Adicionalmente, de acuerdo con Tabatabei et al., (2011), el biodiesel a partir de microalgas tiene las siguientes ventajas:

- i) El potencial de producción de aceite de microalgas es más alto que el de cultivos oleaginosos.
- ii) No tienen efectos adversos en la agricultura tradicional, ya que no se utilizan como alimento y no se cultivan en tierras de cultivo.
- iii) Pueden crecer en ambientes extremos.
- iv) También pueden ser cultivadas utilizando sólo agua de mar, CO_2 y la luz solar, y, por último,
- v) Además de biodiesel, se puede utilizar para la producción de un amplio espectro de los biocombustibles y subproductos.

Por otra parte, otras ventajas de los sistemas de microalgas son que:

- i) Tienen una eficiencia de conversión de fotones superior (como se evidencia por el aumento de los rendimientos de biomasa por hectárea),
- ii) Se pueden cosechar por lotes casi todo el año, proporcionando un suministro fiable y continuo de aceite,

iii) Se puede utilizar agua de mar y corrientes de aguas residuales, lo que reduce en gran medida el uso de agua dulce.
iv) Se puede acoplar la producción de combustible sin emisiones de CO_2 con el secuestro de CO_2 y
v) Se producen biocombustibles no tóxicos y altamente biodegradables.

De lo contrario, existen limitaciones actuales principalmente durante la cosecha y en los procesos técnicos relacionados con el suministro de CO_2 para la producción de alta eficiencia de la biomasa (Schenk et al., 2008) y la gran cantidad de fertilizante requerido durante el cultivo de algas (Ter Veld, 2012). A pesar de eso, hay que recordar que muchos de los productos naturales de microalgas quedan por descubrir.

Aunque las algas se han cultivado comercialmente durante más de 55 años, la ingeniería metabólica ahora parece necesaria con el fin de lograr sus capacidades de procesamiento completos para mejorar el rendimiento y la calidad de los biocombustibles. El estudio de las algas ha generado una gran cantidad de información sobre su fisiología, bioquímica y sus técnicas de cultivo (Rosenberg et al., 2008; Srirangan et al., 2011; Ugwu et al., 2008). En lo que se refiere a la ingeniería genética, estas especies son susceptibles a transformación nuclear, necesaria para el control metabólico y a transformación de cloroplastos, para altos niveles de expresión de la proteína (Rosenberg et al., 2008; León-Bañares et al., 2004; Radakovits et al., 2010). Se confirmó después de un análisis de los posibles impactos ambientales de la producción de biodiesel a partir de microalgas que las microalgas como fuente de energía son excelentes, pero resalta la imperiosa necesidad de reducir el consumo de energía y fertilizantes (Lardon et al., 2009). Mientras que Campbell et al. (2011), señalan la necesidad de una alta tasa de producción para producir biocombustibles puros a precios competitivos, con características de rendimiento excepcionales, es decir, hacer la producción de biodiesel de algas una actividad económicamente atractiva.

El desarrollo de metodologías para la transformación de microalgas ha avanzado significativamente en los últimos 15 años. La modificación genética y las herramientas moleculares han sido desarrolladas para las algas verdes (*Chlorophyta*), rojas (*Rhodophyta*) y marrones (*Phaeophyta*), diatomeas, euglénidos, y dinoflagelados. Más de 30 cepas diferentes de microalgas se han transformado con éxito hasta la fecha (Radakovits et al., 2010). Hay que recordar que ambos orgánulos cloroplasto y el núcleo, contienen genomas individuales que ofrecen la posibilidad de incorporar transgénicos independientes. La mayor parte de los avances logrados en este ámbito se han realizado en la transformación de la alga verde *Chlamydomonas reinhardtii* (Fuhrmann, 2002), por lo que la transformación genética estable ha sido reportada en *Cyanidioschyzon merolae* y *Chlamydomonas reinhardtii* a nivel nuclear (Minoda et al., 2004; Meslet-Cladiere y Vallon, 2011), mientras que a nivel del cloroplasto se ha tenido éxito en

Chlamydomonas reinhardtii y en las especies *Porphyridium* (Takahashi et al., 2007). Se ha afirmado que la transformación genética requiere la permeabilización temporal de la membrana celular con el fin de permitir que las moléculas de ADN exógeno entren en la célula (Rosenberg et al., 2008; León-Bañares et al., 2004). Durante un evento de transformación con éxito, un fragmento de ADN se incorpora en el genoma nuclear o cloroplástico de la célula y la célula permanece viable después, sin embargo, la mayoría de las células mueren como resultado de la ruptura de la membrana celular, a pesar de esto, las posibilidades de aumentar la producción de biocombustibles a través de microalgas genéticamente modificadas muestra ser una tecnología prometedora (Tabatabei et al., 2011). Es importante mencionar que durante la manipulación genética, si el organismo sobrevive a la herida inicial, el gen exógeno puede ser reconocido como extraño y pasará al estado degradado. Por otra parte, el posicionamiento del fragmento de ADN dentro del genoma es arbitrario, por lo que podría tener diversos grados de expresión (Tabatabei et al., 2011; Walker et al., 2005).

Además, los microorganismos fotótrofos capaces de realizar biofotólisis pueden utilizar fuentes baratas y abundantes de carbono, electrones y la energía para el crecimiento y la producción de H_2 (CO_2, agua y luz). El gas hidrógeno (H_2) es a menudo considerado como un combustible renovable. H_2 tiene la más alta densidad de energía en peso de cualquier combustible, y produce sólo H_2O tras la combustión. Sin embargo, cada sistema diferente para la producción de H_2 microbiano tiene ventajas y desventajas que se describen en Work et al. (2012) y Kontur et al. (2012). En biocombustibles microbianos, los principales avances se están realizando vía ingeniería genética para desarrollar productos deseados y mejorar los flujos metabólicos hacia moléculas específicas, pero se debe recordar que hay un riesgo ecológico latente cuando los microorganismos genéticamente modificados se dispersan en el medio ambiente (Fernandez-Luqueño et al., 2011).

Sistemas animales para la producción de bioenergía

Algunos investigadores han estado trabajando con animales para producir bioenergía. Melero et al., (2010), estudiaron las grasas animales no comestibles para producir biocombustibles a través de craqueo catalítico. Sin embargo, Ballesteros et al., (2011), mostraron que los biocombustibles a partir de grasas animales aumentan la emisión de monóxido de carbono, el cual es conocido por sus efectos adversos sobre la salud humana (irrita los ojos y los pulmones). De acuerdo con lo publicado hasta el día de hoy, no hay estudios relacionados con animales modificados genéticamente con el objetivo de aumentar la calidad o cantidad de biomasa, con el fin de mejorar la producción de biocombustibles. Podría ser debido al cumplimiento de mejores prácticas científicas y directrices éticas de la ciencia o porque los sistemas animales para la producción de bioenergía tiene una relación costo-beneficio negativa o baja.

Finalmente, es importante reiterar que un enfoque de evaluación de tecnología integrada y holística debe hacerse, a fin de determinar los posibles efectos de los biocombustibles producidos y desarrollados mediante biotecnología, como una herramienta útil para alcanzar la sostenibilidad. La biomasa vegetal, animal y de microorganismos puede ser utilizada para múltiples formas de bioenergía. Sin embargo, hay que recordar que los biocombustibles tendrán una relación costo-beneficio positivo en el largo plazo si el origen de la biomasa es a partir de seres vivos seleccionados por métodos tradicionales o de aquellos mejorados transgénicamente. Adicionalmente, durante los procesos de producción de biomasa es deseable que estos sean de carbono y nitrógeno-negativos, capaces de eliminar las toxinas y contaminantes ambientales con el fin de mitigar la contaminación del medio ambiente y hacer frente a la crisis de la energía asociada con el agotamiento irreversible de las fuentes tradicionales de combustibles fósiles. Los impactos de la bioenergía en los precios de los alimentos, el crecimiento económico, la seguridad energética, la deforestación, el uso del suelo y el cambio climático son complejos y multifacéticos. Por otra parte, estos impactos son ampliamente dependientes de la fuente de materia prima, sus métodos de producción y su ubicación geográfica. Hoy en día existen considerables avances biotecnológicos para mejorar el rendimiento y la calidad de los biocombustibles, pero los impactos ambientales y el desarrollo sostenible deben tenerse en cuenta.

I.2 Justificación

El creciente desarrollo de los bioenergéticos en el mundo ofrece una fuente alternativa de energía “limpia”, sin embargo, la bioenergía moderna presenta claramente un amplio rango de oportunidades, pero también acarrea muchas desventajas y riesgos. Pocas investigaciones han considerado el impacto de las bioenergías en los sectores social, económico y ambiental. Además, sabemos que los tipos de impacto así como su intensidad varían en función de la fuente de biomasa, el tipo de biomasa, el uso de los subproductos, etc. El desarrollo de nuevas industrias de bioenergía podría proporcionar energía a millones de personas que actualmente carecen de ella y al mismo tiempo, generar ingresos y crear empleos en las zonas más pobres del mundo pero, aún no sabemos con certeza cómo y cuánto se contamina el ambiente durante la producción de biomasa, su proceso y su uso como bioenergético. Tampoco sabemos cómo se comportará el precio de los alimentos si la producción de bioenergéticos se hace intensiva y a nivel industrial. Además, no se han reportado cálculos sobre el uso de fertilizantes y pesticidas, ni su efecto en el suelo y agua durante o posterior a la producción de biomasa.

I.3 Hipótesis

A nivel mundial se ha impulsado el uso de las bioenergías tomando en cuenta de manera aislada su impacto social, económico y ambiental, por lo que es necesario considerar enfoques holísticos que nos permitan lograr la transición hacia un desarrollo sustentable.

I.4 Objetivo General

Conocer el efecto potencial que podría tener la generación y uso de bioenergías a gran escala sobre indicadores sociales, económicos y ambientales.

I.5 Objetivos particulares

1. Determinar las principales fuentes de biomasa;
2. Conocer los indicadores sociales, económicos y ambientales más relevantes, ligados a los bioenergéticos y
3. Determinar los retos tecnológicos que implica el uso de bioenergéticos a gran escala.

II. Materiales y Métodos

Realizamos una revisión de literatura con ISI web of knowledge (http://apps.webofknowledge.com/WOS_GeneralSearch_input.do?highlighted_tab=WOS&product=WOS&last_prod=WOS&SID=3A4pHgE52e7@n@9In64&search_mode=GeneralSearch). Nosotros seleccionamos únicamente artículos publicados durante los últimos cinco años. Los tópicos seleccionados fueron "bioenergy", "review", "biotechnology" y "biofuels". Los estudios seleccionados fueron aquellos que consideraban aspectos como carbono neutral, carbono negativo, sustentabilidad, contaminación e impacto ambiental. Adicionalmente, también consultamos algunos manuscritos citados en los artículos que nos arrojó la base de datos. Cada manuscrito se leyó a detalle y se realizó un registro incluyendo el tópico, el objetivo y las conclusiones de cada artículo. Posteriormente, se realizó una compilación de los resultados de todos los estudios y se procedió a redactar las principales secciones de un manuscrito. Sin embargo, consideramos que la revisión había quedado muy extensa, por lo que se decidió dividir el manuscrito original en dos artículos, de los cuales uno está publicado y otro está en revisión (R1).

III. Resultados y discusión

Con el objeto de evitar la reiteración de la información, los resultados y discusión están en el apartado correspondiente en cada artículo.

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Scientific Research and Essays

Review

How green is bioenergy? A review on myths, challenges, biotechnology progress and emerging possibilities

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Bioenergy is a very heterogeneous aggregation of different feedstocks, conversion technologies and end-uses, and are often promoted as a "green" alternative to fossil fuels. In the case of fossil fuels, extraction energy costs would become higher than the actual energy yield due to increased energy costs for research, deep drilling, as well as to the lower quality and accessibility of the still available oil storages. The objective of this manuscript is to discuss the main myths, challenges, biotechnology progress and emerging possibilities about the bioenergy sources throughout the world. Bioenergy production systems are sometimes claimed to be able to fill the future fossil fuel shortages as well as to decrease carbon dioxide emissions and global warming. The success of bioenergy production depends on the quantity and quality of biomass available and the ability to cost effectively utilize it for energy production. The global effects of the development of biofuel production will depend crucially on where and how feedstocks are produced. Advances in biotechnology are needed to develop bioenergy crops more adapted to adverse environmental conditions with higher growth rate and high caloric value. Life-cycle analysis and evaluation of external costs are important for assessing the social and environmental advantages and disadvantages of bioenergy system.

Key words: Renewable energy, biomass, biofuel, environment, fossil fuel.

INTRODUCTION

Renewable energy is the energy generated from natural resources such as water, sunlight, wind, rain, tides, waves, geothermal and biomass sources e.g. micro-organisms, plants, manure, sludge, and domestic organic wastes. Renewable energy sources are continually and naturally replenished in a short period of time. Solar cells, wind turbines, biofuels and other emerging renewable energy technologies are poised to become major energy sources throughout the world. Renewable energy has an important role in the industry, business and households for providing modern energy access to the billions of people in developing countries, as they continue to depend more on traditional sources of energy. Worldwide there is a growing interest in the use of solid, liquid and gaseous biofuels for energy purposes. There are various reasons for this, such as: i) political benefits (for instance, the reduction of the dependency on imported oil), ii) employment creation (biomass fuel create up to 20 times more employment than coal, gas and oil, and iii) environmental benefits such as mitigation of greenhouse

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gas emissions, reduction of acid rain and soil improvements (van Loo and Koppejan, 2008). Fossil fuels provide 85% of the US energy requirements, a figure that is similar in most countries (Scheller et al., 2010). This situation is not sustainable for several reasons; oil reserves are limited, and the increasing use of oil and coal leads to ever increasing CO_2 emissions, which carry the risk of climate change (Scheller et al., 2010). Energy demands are increasing with population growth and economic development. The use of fossil fuels is now widely accepted as unsustainable due to depleting resources and the accumulation of greenhouse gases in the environment that have already exceeded the "dangerously high" threshold of 450 ppm CO_2-e (Singh et al., 2011). To achieve environmental and economic sustainability, fuel production processes are required that are not only renewable, but also capable of sequestering atmospheric CO_2. Biofuels are therefore rapidly being developed because it is a wide area which produces energy such as power, biogas, biodiesel and bioethanol.

Some 1.5 billion people worldwide still lack access to electricity, and approximately 2.6 billion are reliant on wood, straw, charcoal, or dung for cooking their daily meals (REN21, 2010), which shows that a cheap and friendly environmental source of energy is necessary. Production of renewable energy, particularly from bio-mass, can provide economic development and employment opportunities, especially in rural areas, that otherwise have limited opportunities for economic growth.

It is well known that bioenergy have gained wide acceptance among policy markets, scientists, environ-mentalist, agricultural entrepreneurs and the general public. They are increasingly seen as a contribution towards the solution of many problems at one, ranging from the greenhouse effect to the increasing oil prices, energy dependency and rural development (Russi, 2008).

However, a careful analysis of all the related literature reveals that there is no consensus regarding the biomass potential among the researchers, but rather their assessments differ strongly. One of the most critical bottlenecks in increased biomass utilization for energy production is the cost of its logistics operations. So research is further needed to develop better methods for producing bioenergy as well as better ways of assessing and verifying the environmental performance of biofuels. Fundamental research is required to deal with the uncertainties and missing elements in current approaches for bioenergy production. The objective of this manuscript is to discuss the main myths, challenges, biotechnology progress and emerging possibilities about the bioenergy sources throughout the world.

A literature search was undertaken using the ISI web of knowledge database (http://apps.webofknowledge.com/WOS_GeneralSearch_input.do?highlighted_tab=WOS&product=WOS&last_prod=WOS&SID=3A4pHgE52e7@n@9In64&search_mode=GeneralSearch) in which only papers published during the last five years were selected. The topic selected were "bioenergy", "review", "biotechnology" and "biofuels".

Studies from the literature which dealt either with neutral-carbon, negative-carbon, sustainability or environmental impacts, in relation to biofuels or bioenergy were selected. Each manuscript was read in detail and recorded in our database along with its topic, objective and conclusion. Compilations of the results from the different studies were made and the main sections of this manuscript were written.

DISCUSSION

Air, greenhouse gas emissions and bioenergy production

Biofuel is the fuel derived from organic matter either directly from plants or indirectly from agricultural, commercial, domestic and industrial wastes. However, biofuels will be a viable alternative only if they provide a net energy gain, have environmental benefits, be economically competitive, and be producible in large quantities without reducing food supplies (Hill et al., 2006).

Biomass encompasses vegetation, energy crops, as well as biosolids, animal, forestry and agricultural residues, the organic fraction of municipal waste and certain types of industrial wastes. Biomass can be obtained in two ways, either from the residues or from the dedicated energy crops. Biomass residues and wastes are materials of biological origin arising from the by-products and wastes from the agriculture, forestry, forest or agricultural industries, and households. Dedicated crops are grown for energy and an ideal energy crop has efficient solar energy conversion resulting in high yields, needs low agrochemical inputs, has a low water requirement and has low moisture levels at harvest. It is generally considered as a carbon neutral source of energy, as during conversion and combustion roughly the same amount of CO_2 is emitted as was absorbed during the feedstock growth. Its appeal is due to its potential worldwide availability, its conversion efficiency and its ability to be produced and consumed on a CO_2-neutral basis (Cebrucean et al., 2010). Such waste-to-energy plants offer both generation of clean electric power and environmentally safe waste management and disposal (Iakovou et al., 2010). Many research efforts document the current and potential role of biomass in the future global energy supply (Parikka, 2004; Yamamoto et al., 2001). Theoretically, the total bio-energy contribution (combined in descending order of theoretical potential by agricultural, forest, animal residues and organic wastes) could be as high as 1100 EJ, exceeding the current global energy use of 410 EJ (Hoogwijk et al., 2003). Berndes et al. (2003) further reinforce this potential of biomass in the future global energy supply by analyzing earlier studies on the subject.

Recent awareness of CO_2 emissions has resulted in a shift from less environmental friendly fossil fuels to renewable and sustainable energy alternatives (Gil et al., 2010). Among these, biomass is considered to be one of the few viable replacement options (Munir et al., 2009). Biomass can be grown in a sustainable way through a cyclical process of fixation and release of CO_2, thereby mitigating global warming problems (McKendry, 2002). Biomass fixes CO_2 in the form of lignocellulosics during photosynthesis, and the CO_2 emitted from the combustion of these materials makes no net contribution to the accumulation of CO_2 in the atmosphere or to the greenhouse effect.

Converting rainforests, peatlands, savannas, or grasslands to produce food crop–based biofuels in Brazil, Southeast Asia, and the United States creates a "biofuel carbon debt" by releasing 17 to 420 times more CO_2 than the annual greenhouse gas (GHG) reductions that these biofuels would provide by displacing fossil fuels (Fargione et al., 2008). In contrast, biofuels made from waste biomass or from biomass grown on degraded and abandoned agricultural lands planted with perennials incur little or no carbon debt and can offer immediate and sustained GHG advantages (Fargione et al., 2008). However, it is well known that some crops cultivated with organic amendments or organic wastes increases the CO_2 and N_2O concentrations in the atmosphere (Lopez-Valdez et al., 2011; Fernandez-Luqueno et al., 2010), while it has been reported that the use of biodiesel in engines decrease significantly the emission of unburned hydrocarbons, polyaromatic hydrocarbons and soot, particulate matters, carbon monoxide, carbon dioxide and sulfur dioxide but the NO_x, emissions is more with biodiesel (Palit et al., 2011).

It has been reported that assuming the direct substitution and that the electricity generation from energy crops releases zero emissions; the avoided emissions from the investment under analysis may be computed as 17981 ton CO_2 equivalent/year (Carneiro and Ferreira, 2012). However, if the CO_2 emissions are accounting during the whole agriculture system, there will be a CO_2 net emission, that is, after an integrated assessment of sustainability of agricultural systems and land use, the bioenergy may come at the expense of greenhouse gases emissions and environmental health.

The carbon sequestration options can be divided into two categories: the enhancement of the natural sinking rates of CO_2, and a direct discharge of anthropogenic CO_2 (Yamasaki, 2003). The relevant sequestration options in the first category include terrestrial sequestration by vegetation, ocean sequestration by fertilization, and an enhancement of the rock weathering process. In the direct discharge options, the CO_2 produced from large point sources, such as thermal power stations, would be captured and separated, then transported and injected either into the ocean or underground (Yamasaki, 2003). Although the sequestration options are less beneficial in terms of cost per unit CO_2, reduction compared to other options, technical developments in sequestration options are necessary for the following reasons: i) A huge potential capacity for carbon sequestration; ii) carbon sequestration enables a continuous use of fossil fuels, which is unavoidable at the moment, before switching to renewable energy sources.

From an environmental perspective there are several concerns that are innate to certain biofuel production systems depending on the biofuel being utilized and the production process itself, for example, in the absence of methanol reclamation equipment, methane is emitted into the atmosphere during the course of biodiesel production, while the net reduction in emissions resulting from the combustion of biodiesel in lieu of petroleum diesel will be less than optimal due to the offset caused by the emission of methane (Fore et al., 2011). Lapuerta et al. (2005) stated that methyl esters obtained from the most interesting Spanish oleaginous crops for energy use - sunflower and Cynaracardunculus- were both used as diesel fuels. The use of these vegetable esters provides a significant reduction on particulate emissions, mainly due to reduced soot and sulphate formation, while on the contrary, no increases in NO_x emissions or reductions on mean particle size were found.

According to the above information, there are not enough evidences about the bioenergy effects on the atmosphere. However, nowadays it is well known that the production, exploitation and waste disposal of bioenergy increases significantly the greenhouse gas emissions.

Bioenergy cropping systems also helps to balance the greenhouse gas emissions, and it depend on various aspects like plant life cycle, yield, feedstock conversion efficiencies, nutrient demand, soil carbon inputs, nitrogen losses, and other characteristics and management practices. Cropping systems with grain crops have higher feedstock conversion C than biomass crops because they lack the co product lignin, which is a source of energy during combustion (Paul et al., 2007). Cellulosic energy crops such as switchgrass and hybrid poplar provided the largest net greenhouse gas sinks, >200 g CO_2e-C $m^{-2}yr^{-1}$ for biomass conversion to ethanol, and >400 g CO_2e-C $m^{-2}yr^{-1}$ for biomass gasification for electricity generation. The life cycle analysis of gasoline and diesel compared with ethanol and biodiesel from corn rotations, reed canary grass and hybrid poplar show a reduced GHG emissions by ~40, ~85 and ~115%, respectively (Paul et al., 2007).

A substantial effort is required in order to get C-neutral biofuels to stimulate large-scale biofuel production. However, as already explained, it has to be remembered that, critics of the growing biofuels market have long environmental worried over its impact on land-use change throughout the world. Although biofuels are often presented as a contribution towards the solution of the problems related to our strong dependency on fossil fuels, additional research may be required in order to understand the true impact of biofuels. In order to offer enough biofuels without harming the environment some approaches linked to the biotechnologies cutting-edge might be studied. Likewise, research advances are needed along many fronts to efficiently and sustainably harness the potential of biofuels as an environmentally friendly source of energy.

The water footprint of bioenergy

Promotion of energy from biomass for reducing greenhouse gas emissions has led to increased usage of freshwater, especially during the cultivation of biomass (Gheewala et al., 2011). This has raised concerns about the increase in water stress, particularly in countries that are already facing water shortages. The current expansion of bioenergy with a view to both mitigate climate change and provide more sustainable energy solutions portends to have significant implications on land and water use. Increases in demand for freshwater may exacerbate the already existing water stress, which, in some regions, is further expected to be compounded due to the effects of climate change, but it has to be remembered that the effects of increased biomass production on water resources may be ameliorated through proper land, water, and agricultural management practices.

Water footprint of bioenergy crops is related to the energy yield of a crop to its actual water use under actual field conditions during the growing season, and depends on crop type, agricultural production system and climate. It shows large variations for similar crop types, depending on the agricultural production systems and climate conditions (Abdullah, 2010). Large-scale bioenergy crop plantations create both opportunities and challenges to the water sector, and much depends on the choice of species, genotypes, location of production, prevailing management practices, and water management options. Many crops (e.g., corn, sugar cane, oil palm) have high water requirements at commercial yield levels and are best suited to high-rainfall tropical areas, unless they can be irrigated (Fraiture et al., 2008). Water requirements of different types of bioenergy crops per unit of energy produced varies largely due to several plant, environmental and management factors.

Evaluation of impacts on water resources will be an important component of any assessment of energy from biomass in the future. It is known that nearly 80% of the world-population is exposed to high levels of threat to water security (Vorosmarty et al., 2010). The rapid expansion of biofuel crops can significantly affect regional hydrological patterns (Subhadra, 2010), while the water footprint of the growing biofuel sector should be factored into discussions about water security (Vorosmarty et al., 2010).

In India, jatropha plants, a biofuel feedstock with a large water footprint (Gerbens-Leenes et al., 2009), are increasingly being cultivated in rural areas so that biomass crops are competing with agricultural crops. Future studies on bioenergy will need to take into consideration the water aspect so that the trade-offs between climate change mitigation and water stress must to be addressed.

Bioenergy and sustainability: A growing challenge

It has been stated that bioenergy could substitute and hence diminish the impact of fossil fuel combustion. However, the significant growth of bioenergy sources might present sustainability challenges because it may jeopardize the yield traits of bioenergy crops. The production of these crops could lead to competition for land with food crops and land use change resulting in environmental and social impacts. Furthermore, the type of the biomass resource and its management will have its own positive and negative impacts on society. Avoiding or mitigating such risk is crucial to the sustainable future of the bioenergy industry. It is very much necessary to understand such risks, innovation of bioenergy systems, and regulatory and industry measures. The harvest of corn stover and production of herbaceous crops as cellulosic feedstocks for alternative biomass purposes have been shown to have significant energy benefits and their conversion to alternative energy sources can help to reduce the dependence on crude oil and net emissions of greenhouse gases (Powers et al., 2011). However, the removal of stover from fields for use as a bioenergy crop can potentially have multiple environmental impacts, with the largest concerns related to soil quality. Without the stover on the fields, there is concern that the soil organic matter cannot be replenished. In addition, the stover also acts as a physical barrier to reduce erosion.

The use of a continuous corn cropping system has significant advantages for maximizing the removal of stover as the cellulosic bioenergy crop is essentially doubled compared to a corn-soybean rotation (Powers et al., 2011). However, the added N required with stover removal relative to the conventional tillage baseline scenario could result in even greater amounts of N discharged and would require more fossil fuel consumption for energy intensive N fertilizer production and application (Lavigne and Powers, 2007). Additionally, continuous crop grown on soils may increase N leaching or N volatilization, soil C loss and soil losses. The efficient growth strategies of the perennial biomass grasses and trees rely on a pattern of partitioning of newly assimilated and recycled C and N between leaves, shoots and roots, resulting from a continually shifting balance between sources and sink throughout the year.

This balance is affected by biotic (pests and diseases) and abiotic stresses, especially water limitation (Karp and Shield, 2008). Achieving a sustainable approach to the production of crops for bioenergy should include aspects of total yield, water quality, soil quality, pollution, greenhouse gas emission, net energy balance, and crop productivity among other parameters.

Bioenergy is a part of complex interlinked system whose sustainability can be evaluated through life cycle analysis (LCA). It is one of the best methodologies to access bioenergy sustainability, by identifying energy and materials used as well as waste and emissions released to the environment; moreover it also allows the identification of opportunities for environmental improvement. The study highlighted on dedicated crops (maize, sorghum, triticale and miscanthus) and manure through anaerobic digestion (AD), and combined heat and power (CHP) generation shows that when addressing environmental sustainability of bioenergy chains, all life cycle phases and subsystems must be carefully considered, as there is no single dominating item, but rather several of them play an important role in the overall sustainability (Blengini et al., 2011). According to them LCA results will be helpful in order to assist public decision makers during the evaluation of new bioenergy projects, and also to improve the overall environmental performance and boost eco-efficiency.

The LCA of ethanol from corn grain and biodiesel from soybeans shows ethanol yields 25% more energy than the energy invested in its production, whereas biodiesel yields 93% more. Compared with ethanol, biodiesel releases just 1.0, 8.3 and 13% of the agricultural nitrogen, phosphorus, and pesticide pollutants, respectively, per net energy gain (Hill et al., 2006). The greenhouse gas emissions are reduced to 12% by the production and combustion of ethanol and 41% by biodiesel. Biodiesel also releases less air pollutants per net energy gain than ethanol. These advantages of biodiesel over ethanol come from lower agricultural inputs and more efficient conversion of feedstocks to fuel (Hill et al., 2006).

Neither biofuel can replace much petroleum without impacting food supplies. Even dedicating all U.S. corn and soybean production to biofuels would meet only 12% of gasoline demand and 6% of diesel demand. Overall, LCA indicate that biomass energy systems can be energy efficient, significantly reduce green house gas emissions relative to fossil energy and provide other environmental benefits.

Russi (2008) evaluated all the consequences implied by meeting the Directive's target in Italy, using both imported and domestically produced biodiesel. Her studies revealed that the advantages in terms of reduction of greenhouse gas emissions, energy dependency and urban pollution would be very modest.

Additionally, she stated that the small benefits would not be enough to offset the huge costs in terms of land requirement.

There are a lot of information about some myths linked to bioenergy (Khan et al., 2007; Wetzstein and Wetzstein, 2011). For example, bioenergy is considered a type of renewable energy because its source, that is, biomass is a replenishable resource. However, Khan et al. (2007) stated that current fertilizer N management practices, if combined with corn stover removal for bioenergy production, the soil C loss is exacerbated. An excellent discussion about myths surrounding biofuels is analyzed by Wetzstein and Wetzstein (2011).

Bioenergy seeks to contribute to a sustainable environment in order to achieve global prosperity. However, additional hard work is necessary to conduct basic and applied research and development to create scientific knowledge and technological solutions to the challenges linked with the quality and bioenergy availability. Furthermore, each bioenergy development projects have to include side effects elsewhere in order to shape a sustainable future.

Biotechnology and bioenergy: developing together to generate opportunities

Applications of biotechnology play an increasingly important role in solving industrial, food and energy crisis. Biotechnology can be applied to the microbes involved in processing biomass to biofuels. Recent genetic modifications and breeding efforts of bioenergy crops aim at improving biomass yield, quality, and conversion efficiency. Improvements in composition and structure of bio-chemicals in bioenergy crops will enable the production of more energy per ton of biomass and will improve its calorific value, green house gas profile, and global climate change potential (Abdullah, 2010). Conversion of lignocellulosic biomass to fermentable sugars represents a major challenge in global efforts to utilize renewable resources in place of fossil fuels to meet rising energy demands (Lynd et al., 2008). Current corn hybrids have been bred to maximize food and feed production, first through millennia of crop selection by early farmers, then through modern plant breeding and most recently via biotechnology (Heaton et al., 2008). Additionally, lignin removal is an important technical issue for paper manufacturing and is the key challenge for the conversion of lignocellulosic feedstock into liquid transportation fuels such as ethanol (Gutiérrez et al., 2012). The economic viability of tree biomass for biofuel production requires improved processing technologies to meet this challenge. Nowadays, recent research advances are improving plantation trees for bioenergy and bioindustry adapted woody feedstock production through improved breeding, biotechnology and establishment of tree plantations (Seguin, 2011), which also offer potential for carbon sequestration and natural forest preservation. There has been a surging interest in optimizing the ability to extract fermentable sugars from plant-derived cellulose, earth's most abundant energy-rich polymer, for the production of bioenergy (Miller and Keller, 2009). The challenges inherent in this process involve complex biological and chemical problems that must be addressed to develop feasible infrastructure and efficient processes for energy production from biomass (Moon et al., 2010).

Regulatory costs and concerns are important considerations that must be made when transgenic plants are released into the environment, especially for commercialization. Both process and product of transgenic plants is regulated by most governments throughout the world. It will be important to assess the activity of the released genetically engineered crops, how they affect the native soil, and how they spread and survive in the environment. Additionally, the loss of genes from the genetically engineered crops and the possible transfer of genes to other crops will have to be investigated. However, a strategy called transgenic mitigation could be effective for some bioenergy crops (Di Fazio et al., 2012; Moon et al., 2010). Linked to a primary gene of interest, a mitigating gene, which is positive or neutral for crops, but negative or deleterious for potential non-transgenic hosts is introduced into the crop (Al-Ahmad et al., 2004). Finally, the most common way of controlling transgene spread is through management practices, including harvesting on short rotations before flowering begins, and / or the use of buffer zones where pollination and seed establishment are prevented, thereby mitigating spread outside the confines of plantations (Di Fazio et al., 2012).

Biofuels derived from microalgae are considered to be a viable alternative energy resource (Dragone et al., 2012). Microalgae are able to produce 15 to 300 times more oil for biodiesel production than traditional crops on an area basis. Furthermore microalgae have a very short harvesting cycle (1 to 10 days) depending on the process, allowing multiple or continuous harvest with significantly increased yields (Dragone et al., 2012). However, algae, being eukaryotic, can be improved by genetic manipulation much less readily than photosynthetic bacteria. Notwithstanding, the genetic manipulation of organisms such as plants, animals, and microorganisms is an available and well-studied technology which could enhance the yield of metabolites and biomass, in order to increase the biofuels availability throughout the world.

There are several important challenges that needs to be addressed successfully, in order to spur the biomass production without introducing environmental, social or economic disbenefits, such as producing enough biomass without incurring serious damage to the environment and to the food-supply system. Likewise, the development of cutting-edge tools in molecular and synthetic biology, process engineering, and in genetic engineering is desirable in order to produce high-quality biomass at high rates without compromising environmental health, food availability and social welfare.

Environmental, economic, and energetic costs of bioenergy

Global demand for food would require raising overall food production by some 70% between 2005 and 2050 (FAO, 2009). However, the advent of biofuels has the potential to change some of the projected trends and cause world demand to be higher, depending mainly on energy prices and government policies (FAO, 2009), while global demand for transportation fuels is expected to increase even more rapidly (USDE, 2006). There is a great need for renewable energy supplies that do not cause significant environmental harm and do not compete with food supply. Food-based biofuels can meet but a small portion of transportation energy needs. Among current food-based biofuels, soybean biodiesel has major advantages over grain ethanol. The analyses of ethanol and biodiesel (Hill et al., 2006) suggest that in general biofuels would provide greater benefits if their biomass feeding stocks were producible with low agricultural input, or were producible on land with low agricultural value and required low-input energy to convert feedstocks to biofuel. Soybean diesel needs only less energy to convert to biodiesel than corn grain ethanol, because soybeans create long-chain triglycerides that are easily expressed from the seed, whereas in ethanol production, corn starches must undergo enzymatic conversion into sugars, yeast fermentation to alcohol, and distillation. Energy conservation and biofuels that are not food-based are likely to be of far greater importance over the longer term. Biofuels such as synfuel hydrocarbons - synthetic fuels or cellulosic ethanol that can be produced on agriculturally marginal lands with minimal fertilizer, pesticide, and fossil energy inputs, or produced with agricultural residues (Perlack et al., 2005), have potential to provide fuel supplies with greater environmental benefits than either petroleum or current foodbased biofuels.

Ulgiati (2001) presented a comprehensive, system-based case study of biofuel production from maize or corn (*Zea mays* L.), as an example of the comprehensive approach that he suggested for any energy crop. He concluded that the biofuel option on a large scale is not a viable alternative based on economic, energy and eMergy (amount of available energy [exergy] of one form [usually solar] that is directly or indirectly required to provide a given flow or storage of exergy or matter) analyses of the case study data and estimated possible improvement of yield and efficiency. This is true for developed countries due to their huge energy demand compared with what biofuel options are able to supply as well as for developing countries due to the low yield of their agriculture and competition for land and water for food production. However, biofuels may contribute to optimizing the energy and resource balance of agricultural, livestock, or industrial production systems at an appropriate scale. Russi (2008) found that producing energy at large-scale has small benefits, while energy farming could be carried out on a large scale with industrialized agricultural techniques, which imply an intensive use of fertilizers and pesticides.

Bioenergy production gives rise to additional pressure on land and freshwater resources. The productivity of food and biomass feedstocks needs to be increased by improving agricultural practices. If bioenergy could be produced from low-input biomass grown on agriculturally marginal land or from waste biomass, it could provide much greater supplies and environmental benefits than food-based biofuels.

Current bioenergy markets, growing as a result of attractive economics, which involve domestic heat supply, large scale industrial and community combined heat and power generation (particularly from low cost feed stocks from forest residues, bagasse, municipal solid waste, etc.) and co-firing in large coal based power plants. Many bioenergy routes can be used to convert a range of raw biomass feedstocks into a final energy product. Technologies for producing heat and power from biomass are already well-developed and fully commercialized, as are first generation routes to biofuels for transport. A wide range of additional conversion technologies are under development, offering prospects of improved efficiencies, lower costs and improved environmental performance. Transport biofuels are currently the fastest growing bioenergy sector, however today they represent only 1.5% of total road transport fuel consumption (IEA-Bioenergy, 2009). They are expected to play an increasing role with second generation biofuels, which is expected to increase in the next decades. Different technologies exist or being developed to produce electricity from biomass. In the transport sector, first generation biofuels (mainly bioethanol from starch and sugar crops and biodiesel from oil crops and residual oils and fats) are widely deployed, but its production costs depend on the feedstock used and on the scale of the plant. First generation biofuels face both social and environmental challenges because it may cause an increase in food price and possibly indirect land use change. These risks lead to the development in advancing next generation processes which depend on non-food biomass e.g. lignocellulosic feedstocks such as organic wastes, forestry residues, high yielding woody or grass energy crops and algae. The use of these feedstocks for second generation biofuel production would significantly decrease the potential pressure on land use; improve GHG emission reductions when compared to first generation biofuels and result in lower environmental and social risk. Further development of bioenergy technologies is needed mainly to improve the efficiency, reliability and sustainability of bioenergy chains. The bioenergy production may increasingly occur in biorefineries where transport biofuels, power, heat, chemicals and other marketable products could all be co-produced from a mix of biomass feedstocks.

Recently, significant breakthroughs have improved the production methods of 2,5-dimethylfuran (DMF). Such advances have attracted attention towards DMF as a potential gasoline alternative. DMF's physicochemical properties are competitive to ethanol. Firstly, its energy density (31.5 MJ/l) is 40% higher than ethanol (23 MJ/l) and much closer to gasoline (35 MJ/l). Secondly, it has a higher boiling point (92°C) than ethanol (78°C), which makes it less volatile and more practical as a liquid fuel for transportation (Binder and Raines, 2009).

Ahlgren et al. (2010) investigated the land use, environmental impact and fossil energy use when using biogas instead of natural gas in the production of nitrogen fertilizers. The biogas was produced from anaerobic digestion of grass and maize. Their calculations showed that 1 ha of agricultural land in south-west Sweden can produce 1.7 metric tons of nitrogen in the form of ammonium nitrate per year from ley grass, or 3.6 ton from maize. The impact on global warming, from cradle to gate, was calculated to be lower when producing nitrogen fertilizer from biomass compared with natural gas. Eutrophication and acidification potential was higher in the biomass scenarios while the greatest advantage of the biomass systems however lies in the potential to reduce agriculture's dependency on fossil fuels. In the biomass scenarios, only 2-4 MJ of primary fossil energy was required, while 35 MJ/kg N was required when utilizing natural gas.

Liao et al. (2011) found that the bioethanol techno-system is not only supported by commercial energy and materials products, but also substantially by solar radiation and the labor and services invested. The bioethanol techno-system contributed to the overall supply of energy/exergy resources, although in a less efficient way than the process by which the Earth system produces fossil fuels. Their results show that bioethanol cannot be simply regarded as a renewable energy resource. Biofuels are widely seen as substitutes for fossil fuels to offset the imminent decline of oil production and to mitigate the emergent increase in GHG emissions. This view is, however, based on too simple an analysis, focusing on only one piece in the whole mosaic of the complex biofuel techno-system, and such partial approaches may easily lead to ideological bias based on political preference.

One of the most important strengths of biomass is the promotion of the development of rural areas, reducing the rural exodus and reinforcement of local industry. Another very important aspect is the possibility of creating jobs predominantly in less favored regions throughout the world. As potential weaknesses the possible use of land that may be needed for food production is frequently referred (Carneiro and Ferreira, 2012). Additionally, it is well known that there is still also a lack of knowledge about energy crops. Therefore, integrated policies for energy, land use and water management are needed. The contribution of bioenergy to meet the global energy demand can be expanded very significantly in the future, providing GHG savings and other environmental benefits, as well as contributing to energy security, improving trade balances, providing opportunities for social and economic development in rural communities, and improving the management of resources and wastes.

With the increase in global human population throughout the world, more land may be needed to produce food for human or animal consumption, which is a potential challenge for bioenergy, whereby bioenergy could be particularly useful in specific situations where lower pollution levels are important, such as mining, protected areas, coastal, marine environments, etc. According to the last statements, bioenergy could be used as a supplement to other energy form but not as a primary source.

Improvement in the areas of better machinery development for growing and harvesting dedicated bioenergy crops, good site preparation and weed elimination are highly influential on the performance of many energy crops (Clarke et al., 2009). There are also crop losses associated with inefficient harvesting/picking up of cut energy crops.

The production and provision of energy from biomass resources as an economically feasible technology, as a developing environmentally friendly, as a facilitator of develop in the rural areas, and at the same time as a provider of social welfare still presents some challenges. Recent trends in the laws and policy of renewable energies are promoting the technological development, the innovation, production, distribution and use of bioenergies. However, today many countries in the world do not have any socio-economic strategies to spur their bioenergetics development. Additionally, in each country, many different issues should be taken into account, not only the energy yield or the economic cost, but also social and environmental factors, prior to launch of a bioenergetics policy. Moreover, the biomass resources must be produced with high environmental standards. Each country in the world has to formulate and implement a number of innovative policies and programmes to promote bioenergy technologies prior to launch of renewable energy programmes.

Biomass: Challenges and opportunities

Biomass is the non-fossilized and biodegradable organic material originated from plants, animals and micro-organisms (Carneiro and Ferreira, 2012). Biomass is a heterogeneous energy source which may be used to meet a variety of energy needs in houses or industries, including generating electricity, heating homes, fuelling vehicles and providing process heat for industrial facilities. Today, biomass supplies some 50 EJ globally, which represents 10% of global annual primary energy consumption (IEA-Bioenergy, 2009).

Biomass source that are already concentrated in one place, often as a sub-product of another process, tend to be cheaper since they require less intensive collecting and treatment procedures and have no production costs. The potential high efficiency of the biomass power plants along with the use of a fuel associated with renewed life cycles and their possible positive social impacts in particular at regional level, turn biomass into an interesting alternative for the bioenergy generation (Carneiro and Ferreira, 2010).

Biomass energy differs from other renewable energies, however, extensively its use is directly tied to the farms, forest and other ecosystems from which biomass feedstocks are obtained. The use of biomass has environmental and social impacts depending on what type of biomass is used, as well as how and where they are produced. In this sense, sustainability refers to choosing management practices that minimize adverse impacts and compliment land management objectives, such as farm preservation, forest stewardship, food production and wildlife management.

One problem associated with the biomass production in the land use issue is the conflict between food production and bioenergy. Many traditional food crops, such as corn, sugar and vegetable oils are also some of the most commonly used energy feedstocks. Furthermore, agricultural land may be shifted from producing food to the production of dedicated energy crops, contributing an increased price to these commodities. Another serious issue associated with biomass production is the greenhouse gas emissions from land management and land use change. These refer to emissions of greenhouse gases (especially CO_2, CH_4 and N_2O) resulting from agricultural inputs (such as fertilizers), management practices and land use changes (when forests, grasslands or other ecosystems are cleared to produce crops) associated with production of biomass. It is also important that biomass markets will add value to biomass products, residues and productive lands. So the development of biomass production also poses these challenges especially agricultural greenhouse gas emissions, the effect of land-use change, and ecosystem impact associated with biomass thinning in forests, and the indirect effects created by changes in markets for biomass feedstocks for food. Bioenergy must increasingly compete with other energy sources. Logistic and infrastructure issues must be addressed, and there is need for further technological innovation leading to more efficient and cleaner conversion of a more diverse range of feedstocks. Further work on these issues is essential so that policies can focus on encouraging sustainable routes and provide confidence to policy makers and the public at large.

The current frontier in the bioprocessing of organic lies in the biorefinery concept where organic waste is considered as a feedstock for the biological production of high value commodities. There is a particular interest in the production of metabolites as renewable, biodegradable substitutes for petrochemical products. According to Clarke and Alibardi (2010), these metabolites include:

1. Lactate, produced by fermenting carbohydrate rich waste using either fungal or bacterial cultures, for the production of polylactate, a plastic constituent;
2. Polyhydroxyalkanotes, particularly polyhydroxybutyrate, which are natural storage polymer of many bacterial species with properties similar to polyethylene and polypropylene and harvestable from mixed cultures fed with organic wastes;
3. Succinate, a valuable and flexible precusor for pharmaceutical, plastic and detergent production, fermentable from carbohydrate rich wastes by selected bacterial species.

The biorefinery concept is also being applied to produce fuels with a higher value than methane. Although methane is the easiest biofuel to produce, there is strong price incentive to consider H_2 and ethanol instead of methane. The ultimate yield of all of these products from an organic feedstock is proportional to the chemical oxygen demand (COD) of the feedstock. This includes H_2 where microbial electrolysis cells can be used to completely convert carbohydrate to H_2 on a COD basis with only minor power input to the cell. H_2 and ethanol have current market prices of approximately 0.6 and 0.3 $US kgCOD^{-1}, respectively, compared to methane with a price of only 0.07 $US kgCOD^{-1}. Meanwhile, it is known that today a hectare of sugarcane can produce about 6,000 L of ethanol with production costs ranging from US$0.25 to 0.30/L (Leite et al., 2009).

As previously said, bioenergy could be used as a supplement to other energy form or mixed with other technologies but not as a primary source. Kobylecki (2011) studied the possibility to cofire lignite with hard coal and biomass during the operation from large-scale circulating fluidized bed boilers (CFB). His experimental results indicated that the CFB technology was, indeed, 'fuel flexible' and the addition of up to 30 wt% of lignite to the hard coal and biomass mixture did not affect the boiler performance and bed hydrodynamics. Agriculture residues such as palm shell are one of the biomass categories that can be utilized for conversion to bio-oil by using pyrolysis process (Abnisa et al., 2011) which is a biotechnological possibility that has been studied during the last months. Additionally, the possibilities of biomass production in the farm sector have been extensively investigated throughout the world (Schindler, 2010; Jasiulewicz, 2010). Nijsen et al. (2012), made a first global, detailed attempt to estimate the bio-energy potential on degraded areas. Depending on crop type, the potential was estimated at 190 EJ year^{-1} worldwide, of which around 25 to 32 EJ year^{-1} on land not classified at the moment as crop or pasture land or as forests. Degraded areas throughout the world may be a promise for bioenergy production with little negative impacts on food production, biodiversity or GHG emissions. Additionally there are important biotechnological advances to improve the quality and increase the biomass production from crops, microorganisms, and forest, among other sources; however, there is still a question that has not been answered: how and how much biomass energy can be environmentally sustainable?

Although biomass-based renewable hydrocarbons are considered to be one of the sources with the highest potential to contribute to the energy needs for both developed and developing economies worldwide, and taking into account that efforts to make biofuels from renewable resources have escalated over the past few years, today the production of bioenergy from different biomass-derived feedstock have been developed. However, it is necessary take into account and facing up social, economic, environmental, and technological challenges involved in the production of bioenergy from biomass.

CONCLUSIONS

There has been a growing interest in the use of biofuels as a sustainable replacement for fossil fuels over recent years, but a holistic approach should be adopted to account for emissions occurring and the environmental impact. As pointed out by the above authors, biomass utilization is increasingly considered as a practical way for sustainable energy supply and long-term environment care around the world, notwithstanding that bioenergy is not as green as it seems. Biofuels may contribute to optimizing the energy and resource balance of agricultural, livestock, or industrial production systems at an appropriate scale. However, it has to be remembered that, although biofuels under certain conditions help to reduce greenhouse gas emissions, the global effects of an expansion of biofuel production will depend crucially on where and how feedstocks are produced. Finally, notwithstanding the benefits of bioenergy are being promoted, the environmental profile is not fully understood. Thus the holistic approaches to natural resource management should be considered because the bioenergy may come at the expense of greenhouse gases emissions and environmental health. Bioenergy could be used as a supplement to other energy form but not as a primary source. Furthermore, each bioenergy development projects have to include side effects elsewhere in order to shape a sustainable future.

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BIOTECHNOLOGIES TO PRODUCE BIOENERGY: A REVIEW OF POSSIBILITIES, CHALLENGUES AND THE WAY FORWARD

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ABSTRACT

Today the biotechnological approaches to improve the yield of biofuels have been well studied, especially in crops and microorganisms but also in animals. During the last decades some technologies have been implemented to produce biofuels but some environmental concerns have not been considered or there are some studies missing. This review discuss the main possibilities and challenges of the third and fourth generations of biofuels and the biotechnological possibilities to release genetically engineered organisms with improved carbon storage and higher yields to produce carbon and nitrogen-negative biofuels and mitigate the environmental pollution. Four generations of biofuels are developing but maybe we are too close to the fifth generation, in which genetically modified carbon and nitrogen-negative organisms could be developed with the ability to mitigate the environmental pollution and increase the availability of high-quality biofuels. An integrated and holistic technology is required to determine the potential effects of produced biofuels, in order to develop new biotechnologies to shape a real transition to a truly sustainable civilization. The biomass to produce biofuels will only be cost-effective in the long run if they are further domesticated or improved transgenically, while biotechnology-based biomass to create carbon and nitrogen-negative biofuels able to remove toxins and environmental contaminants is desirable in order to mitigate the environmental pollution and confront the energy crisis associated with the irreversible depletion of traditional sources of fossil fuels.

Keywords: Biofuels; Genetically engineered organisms; Environmental pollution; Sustainability.

1. Introduction

Biofuels are often promoted as a potentially attractive solution to reducing the carbon emission of the transport sector and addressing energy security concern, while demand for the first generation biofuels continues to grow strongly. However some biofuels have received considerable criticism recently as a result of: i) rising food price, ii) relatively low levels of greenhouse gas (GHG) abatement and even a net increases in GHG emission for some biofuels, based on full life-cycle assessments; iii) high marginal carbon abatement cost (dollars ton^{-1} C avoided); iv) the continuing need for significant governments support and subsides to ensure that biofuels are economically viable, and v) direct and indirect impact on land use change and the related GHG emission. Today there are four generations of biofuels but due to the advances reached in the biotechnology, we could to be too close to the fifth generation.

The first-generation biofuels refer to the fuels that have been derived from sources like starch, sugar, animal fats and vegetable oil. These biofuels are produced using standard technologies. Some of the most popular types of first generation biofuels are: biodiesel, vegetable oil, biogas, bioalcohols and syngas. However it has to be remembered that, for example, biodiesel can be first, second, third, or even fourth generation depending on the feedstock used for its synthesis.

Second-generation biofuels are produced from sustainable feedstock. These raw material options may result in the production of more fuel per unit of agricultural land, while require less chemicals and energy input i.e. a higher yield in terms of net GJ energy produced per hectare land is reached, but however, also increases land-use change, which reduces its environmental and economic feasibility. The main idea behind second generation biofuels is to avoid competition between bioenergy crops production and the production of food crops. First- and second-generation biofuels like ethanol and biodiesel have a number of inherent limitations that make them less than ideal as a long-term replacement for petroleum.

Third-generation derived from microalgae is considered to be a viable alternative energy resource that is devoid of the major drawbacks associated with first and second generation biofuels. The utilization of organic waste and carbon dioxide in flue gases for the production of biomass further increases the sustainability of third generation biofuels, as it minimizes greenhouse gas emissions and disposal problems.

Fourth-generation technology combines genetically optimized feedstocks, which are designed to capture large amounts of carbon, with genomically synthesized microbes, which are made to efficiently make fuels. Key to the process is the capture and sequestration of CO_2, a process that renders fourth-generation biofuels a *carbon negative* source of fuel. Producing fuel directly from carbon dioxide and conversion of biodiesel into gasoline using genetically engineered organisms are both examples of this biofuels generation.

Fifth-generation implies produce biofuels with inputs such as sunlight, waste CO_2, organic wastes, hazardous materials or non-potable water i.e. create carbon and nitrogen-negative biofuels able to remove toxins and environmental contaminants in order to mitigate the environmental pollution and confront the energy crisis associated with the irreversible depletion of traditional sources of fossil fuels.

The objective of this review is to discuss the main possibilities and challenges of the third and fourth generations of biofuels and the biotechnological possibilities to release genetically engineered organisms with improved carbon storage and higher yields to create and release carbon and nitrogen-negative biofuels and mitigate the environmental pollution.

2. Some traditional biofuels

2.1 Methane

In order to produce methane several perennial grasses have been identified as promising energy crops, such as Miscanthus (*Miscanthus × giganteus*), reed canarygrass (*Phalaris arundinacea* L.), and switchgrass (*Panicum virgatum* L.) (Massé *et al*., 2010). Perennial grasses require less nutrient (McLaughlin and Walsh, 1998) and pesticide (Lewandowski *et al*., 2003) inputs than annual crops. The C4 grasses have been recognized as more promising energy crops than C3 grasses because of a more efficient photosynthetic pathway (Lewandowski *et al*., 2003). C4 switchgrass has additional advantages such as superior above-ground biomass yield across a wide geographical range, adaptability to marginal quality land, and low water and nutrient requirements (Heaton *et al*., 2008; Wright and Turhollow, 2010). Moreover, switchgrass roots enhance soil structural stability and require relatively low inputs of energy, water and agrochemicals per unit of energy produced (McLaughlin and Walsh, 1998). Methane yield of crops can be assessed intrinsically, i.e. in liters of gas per unit of biomass (Amon *et al*., 2007; Amon *et al*., 2010) but it should also be estimated per unit area because crop yield varies among plant species, varieties and geographical location (Weilang, 2003; Amon *et al*., 2007). It is well known that anaerobic digestion of organic waste and residues combines both sustainable treatment and renewable energy production. However, it has to be remembered that some substrates such as lignocellulosic materials are resistant to anaerobic digestion and can be converted into biogas only to a low extent so pretreatment methods are used to improve the biogas yield (Bruni *et al*., 2010; Carrere *et al*., 2010). The low susceptibility of these materials to conversion into biogas is a result of their composition and structure. Lignocellulose is the complex and rigid matrix of plant cells, it is resistant to enzymatic attack because of the tight association between lignin, cellulose and hemicellulose. Cellulose and hemicelluloses can be degraded in biogas processes. Lignin can however not be degraded under anaerobic conditions. Anaerobic digestion provides several advantages, including low sludge production, low energy consumption, waste stabilization and, more significantly, biogas recovery (Speece, 1996; Mata-Alvarez *et al*., 2000). Recently, biomethanation from organic waste has come into the spotlight particularly due to the active research being conducted in alternative energy fields for reasons of energy security, diversity, and sustainability. Moreover, the anaerobic digestion of biomass is a widely commercialized technology, in contrast to other energy sources such as solar, wind, or geothermal (Jeong *et al*., 2010). Currently, there is a global effort to develop alternative fuels which began some decades ago throughout four generations (Fig. 1), while nowadays dozens of biological feedstock sources have been suggested to meet this effort, and the success of their implementation has varied (Beal *et al*., 2011). Additionally, biotechnological possibilities to increase the yield of crops in order to produce biofuels are discussed below.

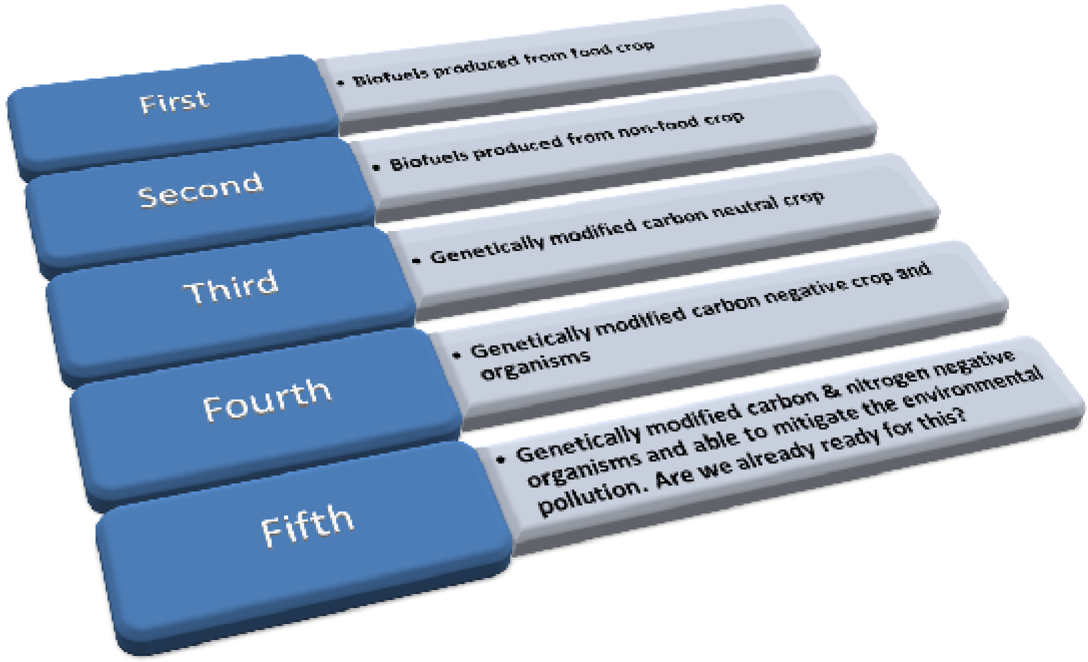

Fig. 1 Four generational categories of biofuels during the last decades and a fifth generation, maybe it has already begun.

2.2 Biodiesel

Biodiesel is a renewable energy produced from algae, seeds, legumes, and from many other sources. Biodiesel is currently produced from oil synthesized by energy crops that harvest the sun's energy and store it as chemical energy. It has to be remembered that the term energy crops can be used both for biomass crops that simply provide high output of biomass per hectare for low inputs, and for those that provide specific products that can be converted into other biofuels such as sugar or starch for bioethanol by fermentation ,or into vegetable oil for biodiesel by transesterification. Biodiesel presents a route for renewable and carbon-neutral fuel production. However, current supplies from oil crops and animal fats account for only approximately 0.3% of the current demand for transport fuels. Increasing biofuel production on arable land could have severe consequences for global food supply. In contrast, producing biodiesel from algae is widely regarded as one of the most efficient ways of generating biofuels and also appears to represent the only current renewable source of oil that could meet the global demand for transport fuels.

The use of lignocellulosic biomass for fuels is limited by several factors. Firstly, biomass is recalcitrant to degradation resulting in large costs and energy consumption in the processes needed to convert biomass into simple molecules that can be used for fuel production. Secondly, fuel production from sugars by current technologies is mostly limited to ethanol, which does not address all the requirements of the transportation sector, while first-generation biofuels are already being produced in significant commercial quantities in a number of countries.

Critical political, economic and environmental security concerns are increasingly linked to petroleum dependence. The extent to which biomass fuels can displace net emissions of carbon dioxide will depend on the efficiency with which they can be produced and used. Thus finding alternatives to petroleum has become a high priority worldwide. A proposed solution to this problem is take advantage of biofuels such as bioethanol, biogas, biodiesel, biohydrogen, etc. derived from microbial and plant biomass. However, it would be recorded that plants have not been domesticated for modern biofuel production, and the quickest, most efficient, and often, the only way to convert plants to biofuel feedstocks is biotechnologically. According to the above statements, it is possible to increase the bioenergy availability but interdisciplinary and multidisciplinary job is necessary in order to develop new technologies to face the energy scarcity. So, the genetic manipulation of organisms such as plants, animals, and microorganisms is an available and well-studied technology which could enhance the yield of metabolites and biomass, in order to increase the biofuels availability throughout the world.

2.3 Power and heat

Renewable energy produced from other sources, or by direct combustion of biomass, lead to generation of electricity, which can be used for many different purposes. However, for transportation, it is essential to have a supply of liquid fuels that meets the requirements of combustion engines used in cars, trucks, airplanes, and ships. In a longer perspective, it may be possible to develop electric cars for much of the short distance transport needs. However, no alternative to liquid fuels is realistic for truck, ship, and airplane transport. The co-firing of biomass with coal in conventional coal-fired boilers can provide a reasonable attractive option for the utilization of biomass for the generation of power and in some case heat. Co-firing makes use of the extensive infrastructure associated with the existing fossil fuel-based power systems, and requires only relatively modest additional capital investment. In most countries, the co-firing of biomass is one of the most economic technologies available for providing significant CO_2 reduction (Van Loo and Koppejan, 2008). Therefore, co-firing biomass with fossil fuels is emerging as a viable option for promoting the use of low quality renewable biomass fuels including energy crops (Lawrence *et al*., 2009; Basu *et al*., 2011; McLlveen-Wright *et al*., 2011). Additionally, biomass has been considered as an alternative fuel to firing coal in utility boilers because of its vast availability and renewable nature (Zhou *et al*., 2010).

Most prior studies have found that substituting biofuels for gasoline will reduce greenhouse gases because biofuels sequester carbon through the growth of the feedstock. These analyses have failed to count the carbon emissions that occur as farmers worldwide respond to higher prices and convert forest and grassland to new cropland to replace the grain (or cropland) diverted to biofuels. By using a worldwide agricultural model to estimate emissions from land-use change, (Searchinger *et al*., 2008) found that corn-based ethanol, instead of producing a 20% savings, nearly doubles greenhouse emissions over 30 years and increases greenhouse gases for 167 years. Biofuels from switchgrass, if grown on U.S. corn lands, increase emissions by 50%. This result raises concerns about large biofuel mandates and highlights the value of using waste products (Searchinger *et al*., 2008).

Nowadays there are many technologies for their possible use with biomass, such as combustion, pyrolysis, gasification and liquefaction. Co-combustion is also one of the most promising options for application with renewable fuels. There are several reasons to blend biomass with coal or with other types of fuel prior to burning. The co-combustion of coal-biomass blends will help to reduce the consumption of fossil fuels. Sometimes biofuel products are mixed with coal to achieve better control of the burning. In co-combustion processes, a volatile matter content greater than 35% is sought in order to provide a stable flame (Biagini *et al*., 2002), which could be attained by using biomass. The ash deposition and fouling problems on hot surfaces, which are commonly encountered in the combustion of biomass can be reduced or altogether eliminated by burning coal-biomass blends (Haykiri-Acma and Yaman, 2008). Furthermore, existing coal-fuelled power plants may continue to be used with very few modifications, and as a final argument, the co-utilization of biomass or waste in existing coal-fired plants is likely to result in a number of environmental, technical and economical benefits.

3. Biotechnological approaches to improve the biofuels production

3.1 Crop production for biofuels generation

Recently, different papers have reviewed the genetically modified crops in order to improve the biofuels crops, e.g., Vega-Sanchez and Ronald (2010), Arruda (2012), Calviño and

Messing (2012), Parry and Hawkesford (2012). It is well known that a number of genomic resources have been developed in several crop species during the last three decades, and provide a platform for exploiting marker technology. To improve important traits in bioenergetics crops, at today there are available several genomic resources, such as: linkage maps, high-throughput sequencing technologies, expression sequence tag databases, genome sequences, DNA chips, targeting induced local lesions in genomes, bacterial artificial chromosome libraries, etc. Childs *et al*. (2012), developed the Biofuel Feedstock Genomic Resource (BFGR) to facilitate genomic-base investigations in poplar, pine, switchgrass, sorghum and maize. BFGR is a database and web-portal that provide high-quality, uniform and integrated functional annotation of gene and transcript assembly sequences from species of interest to lignocellulosic biofuels feedstock scientists.

Today some scientists are working to improve the photosynthetic carbon assimilation in C_3, C_4 o CAM crops, using genetic engineering through single or double gene transformation (Cheng-Jiang *et al*., 2012; Weissmann and Brutnell, 2012; Work *et al*., 2012) to improve the yields and decrease the atmospheric CO_2 concentration. It has to be remembered that there are three types of photosynthetic CO_2 assimilation, and thus three categories of plants: (i) C_3 plants, in which atmospheric CO_2 is assimilated directly through the C_3 photosynthetic pathway e.g., rice (*Oryza sativa* L.), wheat (*Triticum aestivum*), soybean (*Glycine max*), and potato (*Solanum tuberosum*); (ii) C_4 plants evolved the C4 photosynthetic pathway e.g. maize (*Zea mays*) and sugarcane (*Saccharum officinarum*); and (iii) CAM (crassulacean acid metabolism) plants, in which the stomata are closed during the day and open only at night when the temperature decreases and humidity rises. Some researchers are working to increase the biomass yield and reduce the input costs as desirables traits of bioenergy crops (Calviño and Messing, 2012), while recent advances in sequencing methodologies and computational tools promise to accelerate the construction of transcriptional networks.

Nitrogen is a major nutrient which is determinant of crop yield and an essential contributor to quality where protein is a desired trait. However, nitrogen is expensive to produce, distribute and apply, resulting in a large carbon footprint. Since 1909 the Haber-Bosch process converts nitrogen gas into ammonia, which nowadays has allowed for growth of crops used in biofuel manufacturing. It is known that either plant-based or algae-based biofuels require application of nitrogen fertilizer. The reduced nitrogen is assimilated by the plant or algal species to make proteins and nucleic acids, which are not utilized for fuel production. Instead, the high-nitrogen containing residuals are used mainly as animal feed, and eventually result in dispersion of reduced nitrogen on earth. Recycling the ammonia from the protein-rich residuals as a fertilizer for photosynthetic feedstock can close the nitrogen cycle (Huo et al., 2012). Furthermore, research to decrease the N scavenging in soil for growing biofuels crops have not been developed, so that additional investigations are required in order to increase the association (symbiosis) between plants and diazotrophs. Therefore, there could be an increase in the biomass yield i.e. biofuels without additional greenhouse gas emission and pollution generated as an effect of fertilization.

3.2 Microbial and Algal systems for bioenergy generation

Culturing of microalgae as an alternative feedstock for biofuel production has received a lot of attention in recent years due to their fast growth rate and ability to accumulate high quantity of lipid and carbohydrate inside their cells for biodiesel and bioethanol production, respectively. Microalgal systems have the advantage that they can produce a wide range of feedstocks for the production of a number of biofuels including biodiesel, bioethanol, biomethane and biohydrogen. Additionally, microalgae have potential in some areas as nutrition, aquaculture, medicine, environment, cosmetics, and pharmaceuticals (Fig. 2).

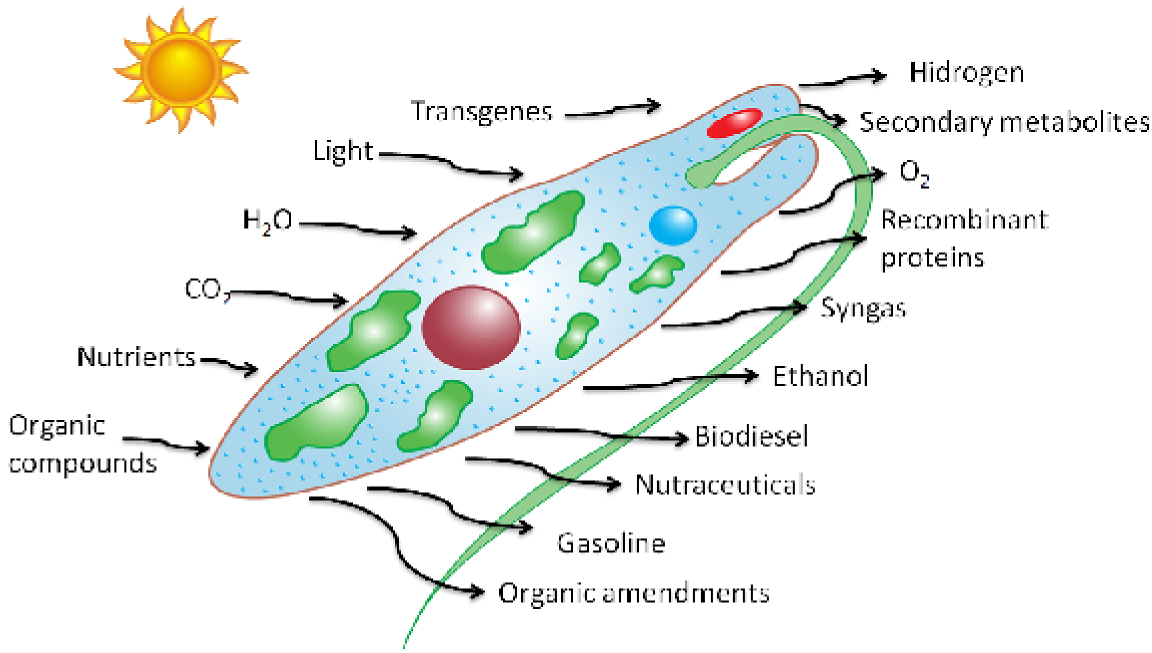

Fig. 2. Microalgal inputs and outputs and their potential to produce some biofuels. Genetic modification is necessary to increase the products and byproducts yield.

Algae are an extremely diverse group of organisms, and it is not surprising that different species of algae produce different compounds that could be used as alternative fuel feedstock. Five commonly studied algal components or products useful for carbon-neutral alternative fuels are: i) lipids for petroleum fuel substitutes e.g. biodiesel, ii) carbohydrates for ethanol, hydrogen, methane via biomass gasification, iii) isoprenoides for gasoline, iv) biomass for syngas, direct combustion, anaerobic digestion, or thermochemical conversion, and v) the direct synthesis of hydrogen gas (Rosenberg *et al.*, 2008; Srirangan, 2011).

Additionally, according to Tabatabei *et al*. (2011), biodiesel from microalgae sustains the following potential advantages:

i) The potential production of oil by microalgae is higher than other oil crops.

ii) They do not have adverse effect on traditional agriculture because they are not used as food and are not cultivated in arable lands.

iii) They can grow in extreme environments.

iv) They can also be cultivated using only seawater, CO_2, and sunlight, and finally,

v) apart from biodiesel, they can be used for production of a broad spectrum of biofuels and byproducts.

Moreover, other advantages of microalgal systems are that they:

i) Have a higher photon conversion efficiency (as evidenced by increased biomass yields per hectare),

ii) Can be harvested batch-wise nearly all-year-round, providing a reliable and continuous supply of oil,

iii) Can utilize salt and waste water streams, thereby greatly reducing freshwater use.

iv) Can couple CO_2-neutral fuel production with CO_2 sequestration, and

v) Produce non-toxic and highly biodegradable biofuels.

Otherwise, current limitations exist mainly during the harvesting and in technical processes linked with the supply of CO2 for high-efficiency production of biomass (Schenk *et al*., 2008) and the great amount of fertilizer required during the algal cultivation (Ter Veld, 2012). Despite that, it has to be remembered that many microalgal natural products remain to be discovered.

Although algae have been commercially cultivated for over 55 years, metabolic engineering now seems necessary in order to achieve their full processing capabilities to improve the yield and quality of biofuels. The study of algae has generated a wealth of information concerning their physiology, biochemistry, and cultivation [40, 41, 45]. In regard to genetic engineering, these species are amenable to firstly, nuclear transformation, necessary for metabolic control, secondly, chloroplastic transformation, for high levels of protein expression, and thirdly, more straightforward approaches to genetic modification compared to higher plants [40, 46, 47].

It was confirmed after an analysis of the potential environmental impacts of biodiesel production from microalgae that the microalgae as an energy source are excellent, but highlights the imperative necessity of decreasing the energy and fertilizer consumption [48]. While [49] highlighted the need for a high-production rate to produce pure biofuels at competitive prices with exceptional performance characteristics i.e. do the algal-biodiesel production one activity economically attractive.

The conversion pathways that are available (i.e., biochemical conversion, thermochemical conversion, and transesterification) for producing algae-based fuel (e.g., biodiesel, methane, hydrogen, electricity, etc.) have been outlined [50-51]. The most comprehensive evaluation of algal species was orchestrated by the US Department of Energy´s Aquatic Species Program (ASP) to develop microalgae as a source of biodiesel [52]. Throughout this project, the ASP recognized that the key to unlocking profitable commercialization of microalgae lies not only in species selection and optimal cultivation, but also in genetic and metabolic engineering [40].

Due to the absence of cell differentiation, it seems that genetic manipulations of microalgae should be a much simpler system compared with higher plants [42]. In addition, allelic genes are usually absent because of the haploid nature of most vegetative stages of microalgae [53]. It is well known that manipulation of metabolic pathways can redirect cellular function toward the synthesis of preferred products and even expand the processing capabilities of microalgae (Fig. 2).

The development of methodologies for microalgal transformation has advanced significantly in the last 15 years. Genetic modification and molecular tools have been developed for the green (Chlorophyta), red (Rhodophyta), and brown (Phaeophyta) algae; diatoms; euglenids; and dinoflagellates. More than 30 different strains of microalgae have been transformed successfully to date [47]. It has to be remembered that both organelles chloroplast and nucleus, contain individual genome which offer the possibility for independent transgenic incorporation. Most of the advances achieved in this area have been made on transformation of the green algae *Chlamydomonas reinhardtii* [54], for which stable genetic transformation has been reported in *Cyanidioschyzon merolae* and *Chlamydomonas reinhardtii* at nuclear level [55, 56], while at chloroplast level it has been reported in *Chlamydomonas reinhardtii* and in *Porphyridium* species [57]. It has been stated that genetic transformation requires temporary permeabilization of the cell membrane in order to allow exogenous DNA molecules to enter the cell [40,46]. During a successful transformation event, a DNA fragment is incorporated into the cell's nuclear or chloroplastic genome and the cell remains viable afterward; however, most cells die as a result of cell membrane rupture, despite this, the possibilities of increasing biofuels production through microalgal genetic engineering shows the promising role of microalgal [42]. Even during the genetic manipulation, if the organism survives the initial wound, the exogenous gene may be recognized as foreign and become degraded. Furthermore, the positioning of the DNA fragment within the genome is arbitrary, which accounts for varying degrees of expression [42, 58].

The major problems in developing microalgae genetic engineering are the low growth rate of microalgae and also the quantity of gene expression in the microalgae species. A variety of transformation methods have been used to transfer DNA into microalgal cells, for example, particle bombardment, agitation of a cell suspension in the presence of DNA and glass beads, agitation in the presence of DNA and silicon carbide whiskers, electroporation, *Agrobacterium* infection, artificial transposons, viruses, and most recently *Agrobacterium*-mediated transformation [59, 60]. Among those, microprojectile bombardment has been developed for transformation of microalgae chloroplast, but the highest transformation rate has been achieved by the methods electroporation, glass-bead, and also particle bombardment [59].

Today, there are many biotechnological approaches to improve or regulate the genetic expression of microalgae, such as genetic transformation method *Agrobacteruim* mediated, cloning strategies. [61] Developed a novel transformation approach of *Schizochytrium* using the *Agrobacterium tumefaciens* binary vector system, while the majority of the transformants displayed similar biomass and fatty acid production comparing with the wild type strain. This strategy makes it possible to explore genetically modified *Schizochytrium* in order to produce higher fatty acids.

Microalgae offer in fact novel bioenergy systems, characterized by much higher oil yields and much lower water demand than terrestrial biomass. However, the processes currently available are marginal in terms of net energy balance and global warming mitigation, so there is still a long way before microalgae will entail a profitable contribution as source of biofuels. Research and development efforts aimed at improving reactor designs and integrating processes are required. Additionally it has to be address both reaction engineering and product separation schemes [62], but we must not forget that the microalgal genetic manipulation is not yet a well-developed strategy to improve the bioenergy yield and provide a favorable contribution from the point of view of global sustainability.

Additionally, photoautotrophic microbes capable of biophotolysis can utilize cheap and plentiful sources of carbon, electrons, and energy for growth and H_2 production (CO_2, water, and light). Hydrogen gas (H_2) is often touted as a renewable fuel. H_2 has the highest energy density by weight of any fuel, and produces only H_2O upon combustion. However, each different system for microbial H_2 production has advantages and disadvantages which are discussed in [37, 63]. In microbial biofuels, major advances are being made in pathway engineering for desired products and to improve metabolic fluxes toward specific molecules, but therefore, it has to be remembered that there is latent ecological risk when genetically engineered microorganisms are spread in the environment [64].

3.3 Animal systems for bioenergy generation

Some researchers have been working with animals to produce bioenergy. [65] studied nonedible animal fats to produce biofuels via the catalytic cracking. However, [66] shows that biofuels from animal fats increase the carbonyl emission which are ubiquitous in ambient air and well known for their adverse effects on human health e.g. eye and lung irritations. According to our current knowledge there is not a study related with genetically engineered animals with the target of increase the quality or quantity of biomass, in order to improve the biofuels production. It could be due to the commitment to the best practices and ethical guidelines of science or because the animal systems for bioenergy production has a negative or low cost-benefit ratio.

Conclusions

An integrated and holistic technology assessment approach should be made, in order to determine the potential effects of biofuels produced and developed biotechnologically, as a useful tool to reach the sustainability. Plant, animal and microorganism biomass can be used for multiple forms of bioenergy however, it has to be remembered that biofuels will have a positive cost-benefit ratio in the long run whether the biomass source is domesticated or improved transgenically in order to produce biofuels from sustainable feedstock. Additionally, during the biomass growing is desirable to create carbon and nitrogen-negative biofuels able to remove toxins and environmental contaminants in order to mitigate the environmental pollution and confront the energy crisis associated with the irreversible depletion of traditional sources of fossil fuels. The impacts of bioenergy on food prices, economic growth, energy security, deforestation, land use and climate change are complex and multi-faceted. Furthermore, these impacts are widely dependents of the feedstock source, its production methods and its geographic location. Today there are considerable biotechnological advances to improve the yield and quality of biofuels but environmental impacts and sustainable development must to be considered. Additionally some cutting-edge researches at cell, genetic and molecular level are necessaries to improve the feedstock availability, and increase the yield, the quantity, and quality of biofuels, while we must not forget that the process engineering is also an opportunity area for a large-scale bioenergy development. Biotechnology will lead to new opportunities to diversify agriculture, forestry, and bioenergy and enhance the farmers and the biotechnological entrepreneurs role as a reliable suppliers of raw materials to biofuel industry.

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Conclusiones

Ha habido un interés creciente en el uso de los biocombustibles como un sustituto sostenible de los combustibles fósiles en los últimos años, pero un enfoque integral debe ser adoptado para dar cuenta de las emisiones que se producen y el impacto ambiental. La utilización de biomasa se considera cada vez más como una forma práctica para el suministro de energía sostenible y cuidado del medio ambiente a largo plazo en todo el mundo, a pesar de que la bioenergía no es tan verde como parece. Los biocombustibles pueden contribuir a optimizar el balance energético y de recursos de la agricultura, la ganadería, o los sistemas de producción industrial a una escala apropiada. Sin embargo, hay que recordar que, si bien los biocombustibles en determinadas condiciones ayudan a reducir las emisiones de gases de efecto invernadero, los efectos globales de una expansión de la producción de biocombustibles dependerán crucialmente de dónde y cómo se producen las materias primas. A pesar de que se están promoviendo los beneficios de la bioenergía, su impacto sobre el medio ambiente no es completamente entendido por lo que enfoques holísticos deben ser considerados sobre el manejo de los recursos naturales porque la bioenergía puede ser a costa de las emisiones de gases de efecto invernadero y la salud ambiental. La bioenergía podría ser utilizada como un suplemento a otra forma de energía, pero no como una fuente primaria. Además, cada proyecto de desarrollo de bioenergía tienen que incluir los efectos secundarios en otros lugares con el fin de dar forma a un futuro sustentable. Algunas investigaciones de vanguardia a nivel celular, genético y molecular son necesarias para mejorar la disponibilidad de materia prima y aumentar el rendimiento, la cantidad y la calidad de los biocombustibles, mientras que no hay que olvidar que la ingeniería de procesos es también un área de oportunidad para un desarrollo de la bioenergía a gran escala. La biotecnología dará lugar a nuevas oportunidades para diversificar la agricultura, la silvicultura y la bioenergía y mejorar la calidad de vida de los agricultores, por lo que las empresas biotecnológicas jugarán un papel fundamental como proveedores confiables de materias primas para la industria de biocombustibles. Considerando lo anterior, la hipótesis planteada se acepta.

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