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ABSTRACT

Our society needs a sustainable source of energy that can replace fossil fuels as the part of sustainable development. Biofuel is a long term solution to fossil fuels. Because fossil fuels are decreasing fast in the amount & are directly related to the air pollution, water pollution, etc. So the renewable source of energy is required urgently in our environment. Biofuel is defined as the liquid fuel produced from biological sources such as biomass. Biofuel is a renewable and eco-friendly source of energy. The production of biofuel from algae can provide some exclusive advantages such as their rapid growth rate, greenhouse gas fixation ability & high production capacity of lipids.

Keywords: Biofuel, algae, energy, biomass, lipid

INTRODUCTION

Fossil fuels are formed by natural processes, such as anaerobic decomposition of dead organisms. The age of the organisms and it results in fossil fuels is typically millions of year's long process. Fossil fuels contain high percentages of carbon and include petroleum, coal, natural gases, kerosene, etc. Our generation is totally dependent on fossil fuels, because they are the 80% source of energy for industrial purposes, generating electricity, etc.

The fast decreasing amount of fossil fuels leads to search for a renewable and environmental friendly source of energy. Our society urgently needs a sustainable source of energy that can replace the fossil fuels for the sustainable development. Because fossil fuels are decreasing and are directly related to the pollution. They are non-renewable and their burning generates greenhouse gases. The new energy sources include solar and wind energy.

The latest renewable source is biofuel production from algae. Biofuel is defined as the liquid fuel produced from biomass. Biofuel is long term solution to fossil fuels.

The species of algae are suitable for lipid production [1].Different types of algae have different ability to produce different products. Algae have 20%-80% oil contents that could be converted into different types of fuels such as bioethanol, biodiesel, etc. [2]

What is Algae?

Algae are generally microscopic organisms which, live in a wide range of aquatic ecosystem. These organisms use light & carbon dioxide to create the biomass. [16] The species of algae suitable for lipids production. Algae have oil contents that could be converted into different types of fuels like bioethanol, biodiesel, etc. [17]

Classification of Algae

There are two types of algae

Macro Algae

Macro algae are the largest multi cellular algae and called "seaweed". It can grow in variety of ways. These algae are measured in inches. [18]

Micro Algae

Microalgae are unicellular algae and tiny in size. Microalgae are recognized as good potential source for biofuel production. [19]These algae are measured in micrometers. They are grown in suspension within a body of water. They have rapid biomass production. [20]

Microalgae have ability to increase the size by every 24 hours. They have high content of oil and lipid. [21] Microalgae can double in number by every three or one and half hour during the peak growth phase. [22] The micro algal mass culture can be grown on saline water or waste water. [23] These microalgae are gaining the

interest from researchers, entrepreneurs & public. [24] Algal biomass contains three main components: -1) Carbohydrates 2) Proteins 3) Lipids/ Natural oil. The large content of the natural oil made, in the form of Triacylglycerol by microalgae. [25]

CH2O-OCHCH2CH2-----CH2CH3 CHO-OCHCH2CH2-----CH2CH3 CH2O-OCHCH2CH2-----CH2CH3

(STRUCTURE OF TRIACYLGLYCEROL)

Botryococcusbraunii

species Some of diatoms (e.g., Chaetocerosmuelleri) and green microalgae (e.g., Chlorella vulgaris, Chlorococcum littoral, Botryococcusbraunii) have been candidate strains for production of neutral lipids for conversion to various types of biofuels (e.g., biodiesel, kerosene, gasoline) [28, 31-33].B. braunii are unicellular, planktonic, oval-shaped microalgae. The individuals live in a biofilm community that helps contribute to their external hydrocarbon production [31]. The extracellular matrix is a complex, three component systems that all works together to produce the external hydrocarbons as well as protects each cell [82]. The biofilm is so strong so that the cells can withstand drying conditions, as well as extreme temperatures for short amounts of time to allow dispersal to be easier for the organism.

The outer layer of cells in the community contains a high number of chloroplasts. *Botryococcus braunii* blooms tend to be toxic to other microbes, plants, and fish populations within the community. They mainly live in fresh to brackish oligotrophic bodies of water with low nutrient content and thrive when the amount of dissolved inorganic phosphorus in the water increases. They can tolerate temperature from 40° C to -20° C for short amounts of times but prefer around 23° C [30].These are fully photosynthetic organisms, so they require reasonable access to sunlight to carry out its metabolic activities. This microbe has no pathogenic properties.

Microalgae could grow under harsher conditions without needs for nutrients, so that they could be grown in unsuitable areas for agricultural purposes independently of the seasonal weather changes, without competing for arable land use. Compared with other biomass-derived biofuels, algae-based biodiesel is receiving increasing attentions worldwide in the recent years. [17] This process is starting from a critical point which is identification of suitable algal strains. These algal strains possess high constituent amount of lipids and capable of rapid accumulation of large quantities of lipids under various culture conditions. In the past decades, many investigations with the aim of screening the oil producers were implemented in North America, Europe, the Middle East, Australia, as well as in many other parts of the world [29, 30].

Chlorella Species

Microalgae are unicellular photosynthetic organisms that use light energy and carbon dioxide (CO₂), with relatively higher photosynthetic efficiency [34].*Chlorella vulgaris* is one of the most important algae species for producing biofuels because of its fast growth and easy cultivation. However, it is yet to be commercially viable due to its low lipid content [35]. So, increasing the lipid content in algae species is the most important research area that needs to be addressed. The dual requirements of maximizing biomass and lipid production are very tough to achieve.





Genetic engineering or gene technology is used to enhance the production of biofuel contents, oil & the stability of algae. Widjaja *et al.* [28] reported that various research claims that lipid storage in many microalgae was enhanced under environmental stress.

Increasing the lipid content under stress conditions could affect the biomass productivity. If specific culture conditions are applied the productivity of biomass and the productivity of lipid content of *C. vulgaris* can both be enhanced [36]. The variation in the growing conditions can significantly affected the lipid content of *C. vulgaris*.



Figure2. Chlorella vulgaris

Microalgae oil contains high values of palmitic acid, and the concentration of linoleic acid which are required for biodiesel production [39].Microalgae biofuels are non-toxic, highly bio-degradable, contain no sulphur and the leftover materials (after extracting the oil) can be used for ethanol production or as soil fertilizer [40].In comparison with other crops, microalgae have high biomass and high lipid productivities per unit of area [13, 14]. Chisti [34] and Amin [15] reported that the demand for fuel in the transportation industry can only be covered by microalgae as a renewable source.

Characteristics of Microalgae

Many green microalgae are cultured under limited nitrogen or high light conditions where, levels of cellular neutral lipid content are increased. These strains of microalgae with higher lipid contents are more suitable feed stocks to produce biofuels. [42]The biomass yield from cultures suspended in growth media with low nitrogen is quite higher than cultures suspended in media containing elevated levels of nitrogen. Compared with cultures exposed to poor intensity of light, microalgae grow under the higher intensity of light to accumulate the greater level of lipid content. [43]. The lipid content could be increased from 0 to 50% on a dry weight basis by following the strategies such as low nitrogen, higher intensity of light. Excessive light energy is potentially harmful that results in increased susceptibility to photooxidative stress. [20]

However, oil-producing microalgae consume carbon and energy to synthesize and accumulate lipids under nitrogen stress. Microalgae grow and double their biomass within 24 hours, and this duration could be as short as 3.5 hours. They are considered to be thallophytes and oldest living organisms. They contain chlorophyll that is primary photosynthetic pigment. The process of photosynthesis within the microorganisms is identical to that of high plants [42]. Furthermore, the cells of microalgae are grown in the form of aqueous suspension. In this way, these cells present more efficient access to CO_2 , water, and other nutrients. Pigment types, cell wall constituents and storage product's chemical nature are all the main criteria over which classification of microalgae is carried out [44].

The microalgae are heterotrophic or autotrophic in nature. When they make use of inorganic compounds as the carbon's source, they are considered as autotrophic. Whereas, they are photoautotrophic when they use light as energy source and heterotrophic when organic compounds are used for growth [51].

Some photosynthetic microalgae are mixotrophic. They can combine autotrophy and heterotrophy through photosynthesis [45]. Because it is depending on the transfer of mass from air into the microalgae during the process of photosynthesis within the environment of aquatic growth.

In related to the consistent method for heterologous protein expression, the plastid genome of *Chlamydomonas Reinhardtii* has been successfully transformed. However, this technology established in *Chlamydomonas Reinhardtii* might not be a good biofuel production species and could be applied to other algae species [47].

Advantages of Using Micro Algae to Produce Biofuel

Algae can provide some exclusive advantages such as their rapid growth rate, greenhouse gas fixation ability & high production capacity of lipids. A different type of algae has different ability to produce different products. Biomass production from algae definitely has great potential to deliver clean energy. [44]

Easy Growth Rate

One of the most important advantage of using microalgae as the renewable source is that it can be grown very easily. Wastewaters which normally harmful for plant growth are very effective in growing microalgae. The growth rate of algae is 20 to 30times faster than other crops e.g. *Jatropha*. Growing algae do not require the use of herbicides or pesticides [43].

Basic Source Grows Fast

An alga is characterized by the fast growth, only needing water, sunlight and carbon dioxide to grow. Also, algae does not affect freshwater

sources, can be produced using waste and sea water, are biodegradable and generally has almost no negative environmental impact when biomass is produced from it. [48]

Consumption of Carbon Dioxide

When algae used as fuel feedstock its massive consumption of carbon dioxide which is the main reason for climate change, air pollution. It released to the atmosphere by burning fossil fuels. According to latest researches, producing each gallon of biofuel from algae consumes 13 to 14 kg of the CO2. [49]

Wastewater Treatment

The nutrients for the cultivation of microalgae (mainly nitrogen and phosphorus) can be obtained from liquid effluent wastewater; therefore, besides providing its growth environment, there is the potential possibility of waste effluent treatment.

This could be explored by microalgae farms as a source of income in a way that they could provide the treatment of waste water and obtain the nutrients the algae need.

Valuable Co-Products

Algae can also produce valuable co-products, as proteins and biomass after oil extraction that can be used as animal feed, medicines or fertilizers, or fermented to produce ethanol or methane. [45]Producing micro algal biomass is generally more expensive and technologically challenging than growing crops.

Photosynthetic growth of microalgae requires light, CO2, water and inorganic salts. The temperature regime needs to be controlled strictly.

For most microalgae growth, the temperature generally remains within 20°C to 30°C [20]. The biofuel production must rely on freely available sunlight to reduce the cost of production [54]. The inorganic elements are major elements of Growth medium that must provide for constitute the algal cell.

Essential elements include nitrogen (N), phosphorus (P), iron (Fe) and in some cases silicon (Si) [56]. Microalgae is grown in various aquatic environments, such as fresh and marine water, municipal waste waters, industrial waste waters and animal waste waters if there are adequate amounts of carbon (organic or inorganic), N (urea, ammonium or nitrate), and P as well as other trace elements are present [50]. As compared with fresh and marine waters, waste waters are unique in their chemical profile and physical properties.

The great potential of mass production of algal biomass for biofuel and other applications using waste waters indicated by recent researches [57]. However, wastewater based algae cultivation faces with many challenges including variation of waste water composition due to source, infrastructure, weather conditions, and pre-treatment methods, improper nutrient ratios (e.g., C/N and N/P), high turbidity due to the presence of pigments and suspended solid particles which affects light transmission, and the presence of competing micro flora and toxic compounds, and accumulation of growth inhibiting compounds which is worsened if water is recycled and reused [52].Here are different methods to cultivate microalgae. However, widely two cultivation systems are used which are open ponds and closed system.

METHODOLOGY

Various methods are used for production of biofuel. Following is full process of biofuel production and also cultivation methods are given.

Process of Fuel Production

The fuel production processes mainly consist of four stages which are: -

- Cultivation of microalgae
- Harvesting of microalgae
- Extraction of lipid
- Tran's esterification.
- Separation & Purification

THE BRIEF DESCRIPTION ABOUT BIOFUEL PRODUCTION PROCESS

Cultivation of Microalgae

There are main two methods in algae cultivation methods: (a) Open system (b) Closed system. The other methods include "Algal turf scrubber" and "Hybrid system". The microalgae cultivation systems are exposed to sunlight, artificial light or combination of both. The oil yield using artificial light sources from laboratory scale microalgae cultivation systems is higher than the yield from outdoor microalgae cultivation system using sunlight. Higher oil yield at laboratory scale conditions is primarily due to the continuous availability and stability of artificial light sources used in the cultivation systems. [56]

Harvesting of Microalgae

Harvesting is the process of algae concentration in water. The harvesting cycle of microalgae ranges between 1 to 10 days [52]. Harvesting technologies vary with the expenses of production for an algae farm. Centrifuges are one of the technologies that are used to harvest microalgae for many years. In this process, microalgae biomass is separated from the growing solution depending upon the type of microalgae.

Extraction of lipid

Extraction is used to separate algae fatty acids and lipids. [58]Algae oils have a variety of commercial and industrial uses and are extracted through a wide variety of methods. There are mainly two methods physical extraction and chemical extraction. [56]

Physical Extraction

In the extraction process, the oil must be separated from the rest of the algae and this could be achieved by mechanical crushing. When dried, the oil content is still retained in the algae and the oil can be recovered using an oil press. Osmotic shock is sometimes used to release cellular components, such as oil. Other harvesting and extraction methods that are under development include ultrasound. [61]

Chemical Extraction

The oil can be extracted using chemical solvents but the downside to using solvents for oil extraction are the dangers involved in working with the chemicals. Care must be taken to avoid exposure to vapor and skin contact, either of which can cause serious health damage. Chemical solvents also present an explosion hazard. The inexpensive chemical solvent called hexane is a common choice and it is widely used in the food industry.

Benzene classified as a carcinogen and ether may also be used to separate the oil. In the Soxlet extraction method, oils from the algae are extracted through repeated washing, or percolation, with an organic solvent such as hexane or petroleum ether, under reflux in special glassware. [62]This technique benefits from the fact that the solvent can be reused.

In enzymatic extraction, enzymes are used to degrade the cell walls with water acting as the solvent and this makes the fractionation of the oil much easier. The costs of this extraction process are estimated to be much greater than hexane extraction [63]. The enzymatic extraction can be supported by ultra-sonication. The combination "son enzymatic treatment" causes faster extraction and higher oil yields.

Trans Esterification

Trans esterification is a step include in conversion process. The conversion process is used to produce renewable energy by converting the fraction of algae lipids into "Fatty Acid Methyl Esters" (FAME) and finally into biofuel. Depending on the quality of fatty acid, some conversion methods are performed in a one-step trans esterification process using a base catalyst and methanol reagent. [64]

The trans esterification is the reversible reaction of fat or oil (composed of triglyceride) with an alcohol to form fatty acid alkyl ester & glycerol. Stoichiometrically, the reaction requires a 3M: 1M alcohol to oil ratio, but excess alcohol is (usually methyl alcohol used) added to drive the equilibrium toward the product side. [65]

The reaction occurs stepwise. Triglycerides are first converted to di-glycerides. Then diglycerides are converted to mono glycerides. In the last mono glycerides are converted into glycerol. [66]

Filtration

Filtration is the physical separation process in which the particles in suspension are retained using a filter. The filters are highly efficient and safe in solid-liquid separation process. The filtration is a separation method that is suitable for large microalgae. [68]

THE CULTIVATION METHOD

Microalgae are cultivated in several ways.

There are mainly two systems in algae cultivation methods: -1) Open system2) Closed system. The other methods include "Algal turf scrubber" and "Hybrid system" which are latest methods. In general, an open pond is simply a series of raceways outside, while a photo bioreactor is a sophisticated reactor design which can be placed indoors in a greenhouse, or outdoors. The details of the two systems are described below. [60]

Open System

Open system include "open pond cultivation" method. It is the most widely used system for large scale of microalgae cultivation. Open ponds are the simplest and cheaper systems of microalgae growth. However, other organisms

can contaminate the pond and damage the microalgae. For instance, the dry land is used for the production of microalgae.

It is observed that the growth of microalgae is 20 to 30 times higher as compared to food crops. The Turkey Soley Institute Company has a great achievement of 1.7 kg microalgae/day in 1 m3water media. [68]

The conditions of microalgae cultivation are greatly influenced by its composition and growth characteristics. The microalgae cultivation systems are exposed to sunlight. Solar energy is the cheapest light sources used in most of the commercial microalgae cultivation systems like the open ponds. In spite of, the process efficiency of the outdoor cultivation systems are poor and require huge areas of land. [70]

However, the major drawback of this system is the unstable solar energy supply acting as a limiting factor for application and efficiency of outdoor process. There is a need for further evolution of technologies with higher microalgae growth rates, the capability of outdoor cultivation and with a strong resistant mechanism to minimize the contamination risks in the largescale microalgae cultivation systems [67].

Cultivation of algae in open ponds has been extensively studied. Open ponds can be categorized into natural waters (lakes, lagoons, ponds) and artificial ponds or containers. The most commonly used systems include shallow big ponds, tanks, circular ponds and raceway ponds. One of the major advantages of open ponds is that they are easier to construct and operate than most closed systems.

However, major limitations in open ponds include poor light utilization by the cells, evaporative losses, diffusion of CO2 to the atmosphere, and the requirement potentially of large areas of land. [71]Open ponds are highly contamination vulnerable to bv other microorganisms, such as other algal species or bacteria and open to the elements. Furthermore, contamination by predators and other fastgrowing heterotrophs have restricted the commercial production of algae in open culture systems to only those organisms that can grow under extreme conditions. Also, due to inefficient stirring mechanisms in open cultivation, the mass transfer rates are very poor resulting to low biomass production. [68]

The ponds in which the algae are cultivated are usually what are called the "raceway ponds". In these ponds, the algae, water and nutrients circulate around a racetrack. With paddlewheels providing the flow, algae are kept suspended in the water, and are circulated back to the surface on a regular basis.

The ponds are usually kept shallow because the algae need to be exposed to sunlight, and sunlight can only penetrate the pond water to a limited depth. The ponds are operated in a continuous manner, with CO2 and nutrients being constantly fed to the ponds, while algae-containing water is removed at the other end. Open pond systems are cheaper to construct as the minimum requirement is just a trench or pond. Open ponds have the largest production capacities relative to other systems of comparable cost. [56]

The biggest advantage of these open ponds is their simplicity, resulting in low production costs and low operating costs. The cost of closed system is comparatively more expensive than open system.

While the open pond is indeed the simplest of all the growing techniques, it has some drawbacks owing to the fact that the environment in and around the pond is not completely under control. Bad weather can stunt algae growth. Contamination from strains of bacteria or other outside organisms often results in undesirable species taking over the desired algae growing in the pond.

The water in which the algae grow also must be kept at a certain temperature, which can be difficult to maintain. Another drawback is the uneven light intensity and distribution within the pond. Despite all these, various US government sponsored studies still favour the open ponds based on low cost. [69]Open ponds are the oldest and simplest systems for mass cultivation of microalgae.

In this system, the shallow pond is usually about 1 foot deep; algae are cultured under conditions identical to their natural environment. The pond is designed in a raceway configuration, in which a paddlewheel provides circulation and mixing of the algal cells and nutrients.

The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with plastic to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around bends to minimize space [63].The system is often operated in a continuous mode that is, the fresh feed containing nutrients including nitrogen phosphorus and inorganic salts is added in front

of the paddle wheel. Algal broth is harvested behind the paddle wheel after it has circulated through the loop (Figure 5).Depending on the nutrients required by algal species, a variety of wastewater sources can be used for the algal culture, such as dairy/swine lagoon effluent and municipal wastewater.

For some marine types of microalgae, seawater or water with high salinity can be used. [62]Although open ponds cost less to build and operate than enclosed photo bioreactors, this culture system has its intrinsic disadvantages. Since these are open-air systems, they often experience a lot of water loss due to evaporation.

Thus, microalgae growing in an open pond do not uptake carbon dioxide efficiently, and algal biomass production is limited [54].Biomass productivity is also limited by contamination with unwanted algal species as well as other organisms from feed.

In addition, optimal culture conditions are difficult to maintain in open ponds and recovering the biomass from such a dilute culture is expensive.[64]"Open pond cultivation" which is further classified into: -

- (a) Raceway pond
- (b) Circular pond
- a) Raceway Pond

Raceway ponds are the most commonly used artificial system. These are made up of closed loop, oval shaped recirculation channels having a depth of 0.2 to 0.5m.

Mixing and recirculation are required to stabilize algae growth and productivity. Raceway ponds are built of concrete and compacted earth-lined ponds with white plastic have been also used.

In a continuous production cycle algae broth and nutrient are fed in front of the paddle wheel and circulated through the loop to the harvest extraction point. The paddlewheel is continuously operated to prevent sedimentation [65].The CO2 requirement is fulfilled from the surface air or submerged aerators may be installed to enhance CO2 absorption [66].One of the significant challenges of using raceways is biomass recovery. This challenge has been mitigated to an extent by immobilized cultures or attached algal processes [33].





Figure3. Raceway pond [15]

The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with plastic to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around bends to minimize space. [63] The system is often operated in a continuous mode that is, the fresh feed containing nutrients including nitrogen phosphorus and inorganic salts is added in front of the paddle wheel. Algal broth is harvested behind the paddle wheel after it has circulated through the loop (Figure 3).

b) Circular Pond

Open ponds are typically built in circular configuration. The water is kept in motion, for example by pivoted agitator. The circular open ponds are of 30 -70 cm in depth. They also called as "circular central-pivot ponds". [62]

Advantages and Disadvantages of Open System

Open systems are economically easy to clean up and easy maintenance. Operation cost and maintenance cost of this system are relatively low. It also includes less capital equipment to maintain.

The systems used in pilot projects partially funded by government. The fresh water is not required, microalgae can grow in wastewater. The microalgae harvested from open pond systems are high in oil content. Chlorella vulgaris has ability to grow in open pond system and produce high oil content. Utilization of nonagricultural land and at low energy input, large energy outcome. It is easy to build. Cultivation of microalgae can be made directly in pond. Large ponds have largest production capabilities compared to other system related to costs. [63] There are some disadvantages of this method, which is including poor productivity limited to few strains. The culture gets contaminated by several environmental factors because it is the open pond cultivation method. [72]

Closed System

The cultivation in closed system is carried out through closed tubular reactors, and it is particularly attractive for the reducing risk of contamination. [76]Closed system includes loop system. It is an enclosed system for the cultivation of microalgae. Photo bioreactor system is a closed system. There are many types of photo bioreactor. [74]Enclosing a pond with a transparent or translucent barrier effectively turns it into a greenhouse. This solves many of the problems associated with an open system. It allows more species to be grown; it allows the species that are being grown to stay dominant; and it extends the growing season and in cold region locations if the pond is heated cultivation can be carried out all year round. [74]

Photo Bioreactor

A bioreactor is an installation to produce microorganisms outside their natural but inside an artificial environment. The prefix "photo" particularly describes the bio-reactor's property to cultivate phototrophic microorganisms or organisms which grow on by utilizing light energy. Photo bioreactor or PBR, is the controlled supply of specific environmental conditions for respective species. [71]

The photo bioreactor (PBR) is a translucent bioreactor container incorporating a light source in which algae are grown. As opposed to an open pond system, the photo bioreactor is usually a closed system.

PBR allows much higher growth rates and purity levels anywhere in natural or habitats similar to nature. [73] Since the system is usually closed all the nutrients must be provided by the cultivator. Farming can be in batch involving restocking the reactor after each harvest or continuous operation that requires precise control of all elements to prevent immediate collapse.

For continuous operation correct amount of sterilized water, nutrients, air and carbon dioxide must be provided. Maximum production occurs when the time to exchange one volume of liquid matches the time to double the mass or volume of the algae.

Algae grown in this controlled mode is said to be of higher nutrient content. The photo bioreactor may be made in the form of a tank, polyethylene sleeves or even as a bag. [56]

Flow Description

- From the feeding vessel, the flow progresses to the pump which moderates the flow of the algae into the actual tube. Built into the pump is the CO2 inlet valve.
- The photo bioreactor itself is used to promote biological growth by controlling environmental parameters including light. The tubes are made of acrylic and are designed to have light and dark intervals to enhance the growth rate.

- The photo bioreactor has a built-in cleaning system that internally cleans the tubes without stopping the production.
- After the algae have completed the flow through the photo bioreactor, it passes back to the feeding vessel. As it progresses through the hoses, the oxygen sensors determine how much oxygen has built up in the plant and this oxygen is released in the feeding vessel itself. It is also at this stage that the optical cell density sensor determines the harvesting rate.
- When the algae are ready for harvesting, they pass through the connected filtering system. This filter collects the algae that are ready for processing, while the remaining algae passes back to the feeding vessel.
- The flow continues [78].

The photo reactor system can be sub-classified as: - (a) Vertical photo reactor, (b) flat or horizontal photo reactor, and (c) helical photo reactor. The helical photo reactor is considered the easiest to scale up production. Compared to open ponds, tubular photo bioreactors can give better pH and temperature control, better protection against culture contamination, better mixing, less evaporative loss and higher cell densities [76]. Tubular photo bioreactors are the only type of closed systems used at large scale production of algae [22].

However, each system has relative advantages and disadvantages. One of the significant challenges of using tubular photo bioreactors is biomass recovery. This challenge has been mitigated to an extent by immobilized cultures or attached algal processes [76].

Enclosed photo bioreactors: Enclosed photo bioreactors have been employed to overcome the contamination and evaporation problems encountered in open ponds. These systems are made of transparent materials and generally placed outdoors for illumination by natural light. The cultivation vessels have a large surface area-to-volume ratio [74]. The most widely used photo bioreactor is a tubular design, which has several clear transparent tubes, usually aligned with the sun rays .The tubes are generally less than 10 centimetres in diameter to maximize sunlight penetration.

The medium broth is circulated through a pump to the tubes, where it is exposed to light for photosynthesis, and then back to a reservoir. The algal biomass is prevented from settling by maintaining a highly turbulent flow within the reactor, using either a mechanical pump or an airlift pump. A portion of the algae is usually harvested after the solar collection tubes.

In this way, continuous algal culture is possible. In some photo bioreactors, the tubes are coiled spirals to form what is known as a helical tubular photo bioreactor, but these sometimes require artificial illumination, which adds to the production cost. Therefore, this technology is only used for high-value products, not biodiesel feedstock [54].

The photosynthesis process generates oxygen. In an open-raceway system, this is not a problem as the oxygen is simply returned to the atmosphere. However, in the closed photo bioreactor, the oxygen levels will build up until they inhibit and poison the algae. The culture must periodically be returned to a degassing zone, an area where the algal broth is bubbled with air to remove the excess oxygen. Also, the algae use carbon dioxide, which can cause carbon starvation and an increase in pH. Therefore, carbon dioxide must be fed into the system to successfully cultivate the microalgae on a large scale. Photo bioreactors may require cooling during daylight hours, and the temperature must be regulated at night hours as well. This may be done through heat exchangers, located either in the tubes themselves or in the degassing column [78].

Mass cultivation of micro algal species that lack pronounced environmentally selective advantages might require the use of photo bioreactors. Photo bioreactors are transparent containers or vessels designed to have reduced light path to enhance the amount of available light to the algal cells, and the cultures within are continuously mixed to enhance nutrient distribution and gas exchange.

Photo bioreactors for microalgae production have an optimal thickness of about 2-4 centimetres. The tubular and the flat-plate are the two most common types of micro algal photo bioreactors [77].

All photo bioreactors have large surface to volume ratio (SVR). Because of their widespread availability, tubes long have been used as a basic photo bioreactor material. The geometric configurations of tubular photo bioreactors span a wide range from straight horizontal, straight vertical, helical, to triangular configurations. One of the world's largest photo bioreactor facilities is in a greenhouse in Klutzes, Germany. This facility consists of straight horizontal tubes stacked in vertical fence-like arrays [76].

The facility has a total volume of 700 cubic meters (m3), occupies a total land area of 10,000 m2, and produces 35-41 grams dry weight/m2 per day or 120-140 dry tonnes per year.

Algae wall adhesion, bio fouling, large pressure drop, and gradients in pH, dissolved oxygen, or CO2 can occur along the tube length. These factors are potential disadvantages of tubular photo bioreactors, which might be resolved by innovative engineering designs [66].Flat-plate (or flat-panel) photo bioreactors are transparent rectangular containers (usually vertical or inclined) with a light path of 1-30 centimetres. Flat-plate photo bioreactors mix substrate by vigorous air sparing from the bottom [76].Productivities of algal biomass in photo bioreactors vary with the type of geometric configuration used and the algal species grown. Many novel production systems have been designed and currently are being developed and tested.

Advantages of Enclosed Photo Bioreactors System

The advantages of the enclosed photo bioreactors are obvious. They can overcome the problems of contamination and evaporation encountered in open ponds. The biomass productivity of photo bioreactors can be 13 times greater than that of a traditional raceway pond, on average [66].Harvesting of biomass from photo bioreactors is less expensive than that from a raceway pond, since the typical algal biomass is about 30 times as concentrated as the biomass found in raceways [76]. Cultivation of algae is in controlled circumstances, hence potential for much higher productivity large surface-tovolume ratio. PBRs offer maximum efficiency in using light and therefore greatly improve productivity. Typically, the culture density of algae produced is 10 to 20 times greater than bag culture in which algae culture is done in bags - and can be even greater. Better control of gas transfer. Reduction in evaporation of growth medium. More uniform temperature. Better protection from outside contamination. Space saving - Can be mounted vertically, horizontally or at an angle, indoors or outdoors [15].

Reduced Fouling

Recently available tube self-cleaning mechanisms can dramatically reduce fouling. Covering ponds does offer some of the benefits that are offered by photo bioreactors, but enclosed systems will still provide better control of temperature, light intensity, better control of gas transfer, and larger surface area-to-volume ratio.

An en closed PBR design will enhance commercial algal biomass production by keeping algae genetics pure and reducing the possibility of parasite infestation [58].

Advantages of photo bioreactors include significantly higher micro algal biomass productivity and greater production stability over time than open-pond systems. For example, the volumetric productivity of Nannochlorops is spp. in photo bioreactors could exceed that in open raceways by as much as 16 times.

The risk of biological contamination is much greater in open-pond systems than in closed photo bioreactor systems [79].Except for Spirulina and Dunaliella Salinas, which are cultivated in open systems under highly selective growing conditions, the lack of competitive advantages of many of the micro algal species being tested for biofuel production in open ponds and their susceptibility to culture crashes are concerns [80].

Thus, the low volumetric productivity and susceptibility to contamination could constitute a substantial risk to the economic sustainability of open pond cultivation systems compared to closed photo bioreactor systems [58].

Disadvantages of Enclosed Photo Bioreactors System

However, enclosed photo bioreactors also have some disadvantages. For example, the reactors are more expensive and difficult to scale up. Moreover, light limitation cannot be entirely overcome since light penetration is inversely proportional to the cell concentration [81]. Attachment of cells to the tube walls may also prevent light penetration. Although enclosed systems can enhance the biomass concentration, the growth of microalgae is still suboptimal due to variations in temperature and light intensity [74].Capital cost is very high.This is one of the most important bottlenecks that are hindering the progress of algae fuel industry. Despite higher biomass concentration and better control of culture parameters, data accumulated in the last two decades have shown that the productivity and production cost in some enclosed photo bioreactor systems are not much better than those achievable in open-pond cultures. The technical difficulty in sterilizing these photo bioreactors has hindered their

application for algae culture for specific endproducts such as high value pharmaceutical products [58].

Hybrid System

Hybrid systems are the advanced cultivation system. These are basically need dependent combination of previous algal production technologies to overcome the demerits. These systems can maintain higher algal densities for maximized algal biomass production within less cultivation area. These systems are called with different names depends on the combination and use of technology [42].E.g. closed pond system, photo bioreactor, Turf systems [41, 42]Artificial environment is created to increase yield and decrease the harvesting time of algal production. The major factors that use to control are sunlight, CO2 and nutrients in the system. For an example: algae photo bioreactor is used to smoke stack for more CO2 supply. Artificial lights are used to minimize the harvest time and increase the production. The advantages of each system by using "hybrid" processes that combine two or more methods. These can be small PBRs that inoculate large ponds, larger PBRs used in combination with ponds, or ponds and fermenters used sequentially. The objective of hybrid systems is to maximize the individual advantages of each process [76].

Hybrid Photo Bioreactors

Hybrid systems of algae cultivation involve the integration of various phases of microalgae growth in a single two-stage system. The initial part of algae growth takes place in a closed system (i.e. photo bioreactor), whereas the subsequent ones in the open system. Hence, the hybrid systems include components and design solutions that are characteristic of the various cultivation systems, i.e. open and closed ones [62]. The first phase of the cultivation is realized in a photo bioreactor, which is meant to minimize the hazard associated which the contamination of the cultivation by foreign organisms while the beneficial conditions for cell multiplication are maintained. The second stage of production is designed to expose the cells to the stress of nutrients, which should initiate the synthesis of desired metabolites and lipid products and it is realized in cultivation ponds. Such system can include the production of oil and xanthophyll pigment by Haematococcus pluvialis. [81]Huntley and Redalje [53] reported that the mean amount of oil that can be gained from algae in this manner was equal to above 10 tonnes· ha-1· year-1 and the maximum was even 24 tonnes· ha-1· year-1 [78].

CONCLUSION

Algae are easy to grow & it can produce high vield oil. Algae are very efficient means of producing biofuel. Biofuel is eco-friendly renewable source of energy. Its better option than using fossil fuels. We can provide this new energy source to our next generation. Algae become new interesting subject for researchers and entrepreneurs. Biofuel production from algae can provide some exclusive advantages such as their rapid growth rate, greenhouse gas fixation ability & high production capacity of lipids. Hybrid system and closed cultivation methods are most effective methods. Because of low contamination chances are there in these methods, so that high biomass production of pure culture of biofuel producing microalgae is a beneficial point.

INFERENCES

Prospects of Biofuel from Alga

The given picture is the future imagination about biofuel pump, just like petrol pump which used in present time. Biofuel which is a liquid fuel produced from microalgae have ability to give an energy to cars, bikes, jet plane, etc.

SUMMARY

Different types of algae have different ability to produce different products. Algae have 20%-80% oil contents that could be converted into different types of fuels such as, bio but anol, biodiesel, bio ethanol, etc. Some species such as Botryococcus braunii& chlorella species have good potential for biofuel production. Genetic engineering or gene technology is used to enhance the production of biofuel contents, oil & the stability of algae. This paper reviews the brief discussion on biofuel production process from algae as a renewable source and the detail about cultivation methods. There are mainly two cultivation methods which are open pond system and close system. The close system includes different types of photo bioreactor. Another latest cultivation method is hybrid system.

REFERENCES

- [1] Nnorom Achara, MDPGA, Ministry of Defense, Wethersfield Braintree, United Kingdom, 2012.
- [2] Achara N, Grid Parity and Solar Electricity for Homes, Nature and Science, 2011 9(10), October 2011A.

- [3] Achara N, Wind Turbine Performance –The Betz Limit and Other Factors, Journal of American Science, Vol 7(9), Sept 2011B.
- [4] El-Shimi, H. I., Attia, N. K., El-Sheltawy, S. T., & El-Diwani, G. I. Biodiesel production from Spirulina- platensis microalgae by in-situ transesterification process. Journal of Sustainable Bioenergy Systems, 3(03):224, 2013.
- [5] Mondal, P. Production of biodiesel from algal biomass collected from Solani River using Ultrasonic Technique. International Journal of Renewable Energy Research (IJRER), 4(3): 714-724, 2014.
- [6] Gude, V. G., Patil, P., Martinez-Guerra, E., Deng, S.& Nirmalakhandan, N. Microwave energy potential for biodiesel production. Sustainable Chemical Processes, 1(1):1, 2013.
- [7] Piasecka, A., Krzemińska, I., & Tys, J. Physical methods of micro algal biomass pretreatment. Int. Agro Phys, 28:341-348, 2014.
- [8] Nigam, P.S. and Singh, A. (2011), Production of liquid biofuels from renewable resources, Progress in Energy and Combustion Science, 37(1): 52 68
- [9] Dragone, G., Fernandes, B., Vicente, A.A. and Teixeira, J.A. (2010), Third generation biofuels from microalgae in Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Mendez-Vilas A (ed.), Formatex, 1355-1366
- [10] Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J. and Smith, A.G. (2010), Biodiesel from algae: challenges and prospects, Current Opinion in Biotechnology, 21:277-286.
- [11] Brennan L, Owende P. (2010), Biofuels from microalgae--A review of technologies for production, processing, and extractions of biofuels and coproducts. Renewable and Sustainable Energy Reviews, 14:557-577.
- [12] Chisti Y. (2007), Biodiesel from microalgae. Biotechnology Advances, 25:294-306.
- [13] Achara N, Grid Parity and Solar Electricity for Homes, Nature and Science, 2011 9(10), October 2011A.
- [14] Achara N, Wind Turbine Performance –The Betz Limit and Other Factors, Journal of American Science, Vol 7(9), Sept 2011B.
- [15] IEA, WorldEnergyOutlook2006, International Energy Agency, Paris, France, 2006.
- [16] P. Schenk, S. Thomas-Hall, E. Stephens et al., "Second generation biofuels: high-efficiency microalgae for biodiesel production, "bioenergy Research, vol.1, no.1, pp.20–43, 2008.
- [17] Banerjee, A., Sharma, R., Chisti, Y., & Banerjee, U. C. Botryococcus braunii: a renewable source of hydrocarbons and other chemicals. Critical reviews in biotechnology, 22(3): 245279, 2002.

- [18] Borowitzka, M. A. Commercial production of microalgae: ponds, tanks, tubes and fermenters. Journal of biotechnology, 70(1): 313-321, 1999.
- [19] SHEEHAN J, DUNAHAY T, BENEMANN J, et al. A look back at the US Department of Energy's aquatic species program—Biodiesel from algae. Prepared for the US Department of Energy, Prepared by The National Renewable Energy Laboratory (NREL) [R].
- [20] NREL/TP-580-24190. Golden, CO, 1998.
- [21] CARIOCA J O B, HILUY J, LEAL M, et al. The hard choice for alternative biofuels to diesel in Brazil [J].Biotechnol Adv., 2009, 27(6): 1043–1050.
- [22] VIJAYARAGHAVAN K, HEMANATHAN K. Biodiesel production from freshwater algae [J]. Energy Fuels, 2009, 23: 5448–5453.
- [23] RODOLFI L, ZITTELLI G C, and BASSI N, et al. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photo bioreactor [J]. Biotechnology and Bioengineering, 2009, 102(1): 100–112.
- [24] BLACKBURN S I, DUNSTAN G A, FRAMPTON D M F, et al.
- [25] Australian strain selection and enhancement for biodiesel from algae [J]. Phycologia, 2009, 48(4): 8–9.
- [26] CHENG Y, LU Y, WU Q Y, et al. Alga-based biodiesel production and optimization using sugar cane as the feedstock [J]. Energy Fuels, 2009, 23(8): 4166–4173.
- [27] KONG Q X, LI L, RUAN R, et al. Culture of microalgae Chlamydomonas reinhardtii in wastewater for biomass feedstock production [J]. Applied Biochemistry and Biotechnology, 2010, 160(1): 9–18.
- [28] SHEN Y, YUAN W, MAO E, et al. Heterotrophic culture of chlorella protothecoides in various nitrogen sources for lipid production [J]. Applied Biochemistry and Biotechnology, 2010, 160(6): 1674–1684.
- [29] HU Q, SOMMERFELD M, JARVIS E, et al. Micro algal triacylglycerol as feed stocks for biofuel production: Perspectives and advances [J]. Plant Journal, 2008, 54(4): 621–639.
- [30] Demura, M., Ioki, M., Kawachi, M., Nakajima, N., Watanabe, M.M. (2013). PubMed Database. Characterization of the growth and lipid content of the diatom Chaetocerosmuelleri [J]. J ApplPhycol, 1997, 9(1): 19–24.
- [31] BERBEROGLU H, GOMEZ P S, PILON L. Radiation characteristics of Botryococcusbraunii, Chlorococcumlittorale, and chlorella sp. used for CO2 fixation and biofuel production [J]. Journal of Quantitative Spectroscopy & Radiative Transfer, 2009, 110(17): 1879–1893.
- [32] ILLMAN A M, SCRAGG A H, SHALES S W. Increase in chlorella strains calorific values

when grown in low nitrogen medium [J]. Enzyme and Microbial Technology, 2000, 27 (8): 631–635.

- [33] Chisti, Y. Biodiesel from microalgae. Biotechnol. Adv.2007, 25, 294–306.
- [34] Yusaf, T.; Baker, P.; Hamawand, I.; Noor, M.M. Effect of compress natural gas mixing on the engine performance and emissions. Int. J. Automot. Mech. Eng.2013, 8, 1438–1451.
- [35] Singh, J.; GU, S. Commercialization potential of microalgae for biofuels production. Renew. Sustain. Energy Rev.2010, 14, 2596–2610.
- [36] Mustafa, B. Potential alternatives to edible oils for biodiesel production—A review of current work. Energy Convers. Manga.2011, 52, 1479–1492.
- [37] Miao, X.; Wu, Q. Biodiesel production from heterotrophic micro algal oil. Bio resource. Technol.2006, 97, 841–846.
- [38] Liu, Z.-Y.; Wang, G.-C.; Zhou, B.-C. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioresour. Technol.2008, 99, 4717–4722.
- [39] Widjaja, A.; Chien, C.-C.; Ju, Y.-H. Study of increasing lipid production from fresh water microalgae Chlorella vulgaris. J. Taiwan Inst. Chem. Eng.2009, 40, 13–20.
- [40] Lv, J.-M.; Cheng, L.-H.; Xu, X.-H.; Zhang, L.; Chen, H.-L. Enhanced lipid production of Chlorella vulgaris by adjustment of cultivation conditions. Bioresour. Technol.2010, 101, 6797–6804.
- [41] Converti, A.; Casazza, A.A.; Ortiz, E.Y.; Perego, P.; del Borghi, M. Effect of temperature and nitrogen concentration on the growth and lipid content of Nanno chloropsisoculata and Chlorella vulgaris for biodiesel production. Chem. Eng. Process. Process Intensify.2009, 48, 1146–1151.
- [42] Al-lwayzy, S.H.; Yusaf, T.; Jensen, T. Evaluating tractor performance and exhaust gas emissions using biodiesel from cotton seed oil. IOP Mater. Sci. Eng.2012, 36, 012042.
- [43] Hossain Sharif, A.B.M.; Salleh, A.; Nasrulhaq, A.; Chowdhury, P.; Naqiudden, M. Biodiesel fuel production from algae as renewable energy. Am. J. Biochem. Biotechnol. 2008, 4, 250–245.
- [44] Demirbas, A.; FatihDemirbas, M. Importance of algae oil as a source of biodiesel. Energy Convers. Manga. 2011, 52, 163–170.
- [45] Demirbas, A. Importance of biodiesel as transportation fuel. Energy Policy2007, 35, 4661–4670.
- [46] Amin, S. Review on biofuel oil and gas production processes from microalgae. Energy Convers. Manga. 2009, 50, 1834–1840.
- [47] Demirbas, A. Use of algae as biofuel sources. Energy Convers. Manga.2010, 51, 2738–2749.
- [48] Balta, M.; Balta, H. Progress in biodiesel processing. Appl. Energy2010, 87, 1815–1835.
- [49] Rathore D, Nizami AS, Pant D, Singh A. Key issues in estimating energy and greenhouse gas

savings of biofuels: challenges and perspectives. Biofuel Research Journal 2016; 10:380-393.

- [50] Borowitzka MA, Moheimani NR. Sustainable biofuels from algae. Mitig Adapt Strat Gl 2013; 18:13-25.
- [51] Singh A, Olsen SI. A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels. Appl Energy 2011; 88:3548–3555.
- [52] Gouveia L, Oliveira AC. Microalgae as a raw material for biofuels production. J Ind Microbial Biot 2009; 36:269–274.
- [53] Pittman JK, Dean, AP et al. The potential of sustainable algal biofuel production using wastewater resources. Bio resource Technol 2011; 102:17–25.
- [54] A. Demirbas and M. FatihDemirbas, "Importance of algae oil as a source of biodiesel," Energy Conversion and Management, vol. 52, no. 1, pp. 163–170, 2011.
- [55] Future working.com, 4 February- 2016.
- [56] Tahini S. Gendy*, Seham A. El-Temtamy, process development department, Egyptian petroleum research institute, Nasae city, Cairo, Egypt. 2012.07.001.
- [57] Chisti, Y (2007), Biodiesel from microalgae, Biotechnology Advances, 25:294-306
- [58] Chaumont, D. (1993), Biotechnology of algal biomass production: a review of systems for outdoor mass culture, Journal of Applied Phycology, 5:593-604.
- [59] Borowitzka, M.A. (1999), Commercial production of microalgae: ponds, tanks, tubes and fermenters, Journal of Biotechnology, 70:313-321.
- [60] Borowitzka, M.A. (2005), Culturing microalgae in outdoor ponds In: Andersen RA, eds. Algal Culturing Techniques. Burlington, MA: Elsevier Academic Press, 205-218.
- [61] Pulz, O. (2001), Photo bioreactors: production systems for phototrophic microorganisms, Applied Microbiology and Biotechnology, 57:287293.
- [62] Zhou, W., Chen, P., Min, M., Ma, X., Wang, J., Griffith, R., Hussain, F., Peng, P., Xie, Q., Li, Y.Shi, J., Meng, J., Ruan, R. (2014), Environmentenhancing algal biofuel production using wastewaters, Renewable and Sustainable Energy Reviews, 36: 256-269.
- [63] Molina, G E. (1999), Microalgae, mass culture methods. In: Flickinger MC, Drew SW, editors. Encyclopedia of bioprocess technology: fermentation, bio catalysis and bio separation, vol. 3.Wiley; p. 1753–69.
- [64] G. Pokoo-Aikins, A. Nadim, M. M. El-Halwagi, and V. Mahalec, "Design and analysis of biodiesel production from algae grown through carbon sequestration," Clean Technologies and Environmental Policy, vol. 12, no. 3, pp. 239– 254, 2010.

- [65] Chisti Y, Yan J. Energy from algae: Current status and future trends. Algal biofuels - A status report. Appl Energy 2011; 88:3277–3279.
- [66] Rios SD, Torres CM, Torras C, Salvado J, et al. Microalgae-based biodiesel: Economic analysis of downstream process real-istic scenarios. Bio resource Technol 2013; 136:617–625.
- [67] Bosma R, van Spronsen W A, Tramper J and Wijffels R H. ULTRASOUND A new technique to harvest microalgae, Journal of Applied Phycology 15(2-3) 143-153, 2003
- [68] Cyber lipid, Automatic Soxlet Extraction. cyberlipid.org,
- [69] Hielscher, Biodiesel from Algae Using Ultra sonication, Pienkos PT, Darzins A. The promise and challenges of micro algal-derived biofuels. Biofuel Bio prod Bior 2009; 3:431–440.
- [70] Pittman JK, Dean, AP et al. The potential of sustainable algal biofuel production usingwastewater resources. Bio resource Technol 2011; 102:17–25.
- [71] Sheehan J, Dunahay T, Benemann J, Roessler P, A Look Back at the U.S. Department of Energy's Aquatic Species Program—Biodiesel from Algae, Office of Fuel Development, US Dept. of Energy July 1998.
- [72] Pulz, O. photo bioreactors: production systems for phototrophic microorganisms. Applied microbiology and biotechnology, 57(3): 287-293, 2001.

- [73] Terry, K. L., & Raymond, L. P. System design for the autotrophic production of microalgae. Enzyme and Microbial Technology, 7(10):474-487, 1985.
- [74] Chisti, Y. Biodiesel from microalgae. Biotechnology advances, 25(3): 294-306, 2007. (Figure)
- [75] Hoffmann JP. (1998), Wastewater treatment with suspended and non-suspended algae. Journal of Phycology, 34: 757–763.
- [76] Author Zhiyou Wen, Biological Systems Engineering Department, Virginia Tech., 2017.
- [77] Hoffmann JP. (1998), Wastewater treatment with suspended and no suspended algae. Journal of Phycology, 34: 757–763.
- [78] Mata TM, Martins AA, Caetano NS. (2010), Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev, 14: 217–32.
- [79] M. Huntley, D. Redalje, Mitig. Adapt. Strat. Gl. 12, 4 (2007)
- [80] Weiss, T.L., Roth, R., Goodson, C., Vitha, S., Black, I., Azadi, P., Rusch, J., Holzenburg, A., Devarenne, T.P., Goodenough, U. (2012). Colony Organization in the Green Alga Botryococcus braunii (Race B) Is Specified by a Complex Extracellular Matrix. American Society for Microbiology: Eukaryotic Cell 11(12): p. 1424. Retrieved April 17, 2014. Web. American Society for Microbiology.

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