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REVIEWS

Sustainability of Large-Scale Algal Biofuel Production in India

H.N. Chanakya[1]*, Durga Madhab Mahapatra[1], Sarada Ravi[2], V.S. Chauhan[2] and R. Abitha[1]

Abstract | Algal biofuels are poised to become one of the sustainable sources of biofuels that could potentially replace petroleum derived fuels (PDF) in an environmentally friendly and sustainable world. In many parts of the developing world, algal biofuels as biodiesel and biomethane, like the first generation biofuels of the recent past, will compete for prime land as well as other water and nutrient resources currently used for providing food security. Within India, current analyses suggest that sustainable cultivation of various algal species for biofuels are possible at four locations:

a. In paddy fields as a multi-tier crop (3.6 Mha)
b. In saline brackish region of Kachch (Gujarat; 3.0 Mha),
c. Urban domestic waste water (40 billion L/d), and
d. On fishery deficient seashores (c.3 Mha).

In order to realize a near complete substitution of the current level of fossil fuels by algal biofuels, although feasible through algal biofuels, a whole lot of decentralized algae harvesting and primary processing infrastructure needs to be set up to ensure that algal production, processing and resource recycling can occur with low losses and increased sustainability. The coupling of energy generation with wastewater treatment and consequent nutrient uptake addresses the issues of environment, low emissions, biofuel production and therefore long term sustainability. This paper brings out challenges in the areas of sustainability and sustainable development that are likely to emerge in the three tracts where large scale algal cutivation and processing is feasible.

Keywords: *Algal biofuels; sustainability; wastewater; biomethane; paddy; marine algae.*

1 Introduction

1.1 *Need and justification for algal biofuels*

In a rapidly urbanizing country, coupled with an ever increasing demand for transport and mobility, the demand for petroleum derived fuels (PDF) has increased, greatly to reach an annual requirement of over 150 $\mathrm{Mt_{oe}}$[1,2] (Figures 1 and 2) for which sustainable fuel options and alternatives are required. Globally the search for replacements for PDF has also increased and biofuels such as bioethanol, biodiesel, etc. derived from terrestrial non-edible crops have emerged as short term solutions, however, they carry significant sustainability issues coupled to their use.[3] PDF are considered non-sustainable due to their inherent nature of increasing emissions of green house gases (GHG).[4] Therefore plant derived biofuels are considered renewable because no net emissions of GHG is expected to take place during their production and use, and therefore they are considered C-neutral. Biofuels derived from terrestrial edible and non-edible oilseeds have been used as C-neutral fuels however, they compete for scarce land area with

[1]Centre for Sustainable Technologies, Indian Institute of Science, Bangalore 560 012, India.

[2]Central Food Technological Research Institute, Mysore 570 020, India.
*chanakya@astra.iisc.ernet.in

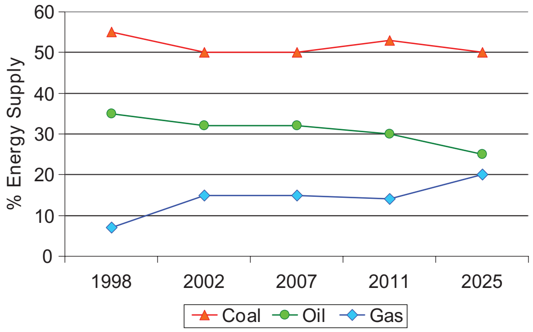

Figure 1: Source-wise distribution of typical fossil fuel derived energy supply in India (Adapted *from Technical Note on Energy, Planning Commission, Govt. of India, 1998–1999*).

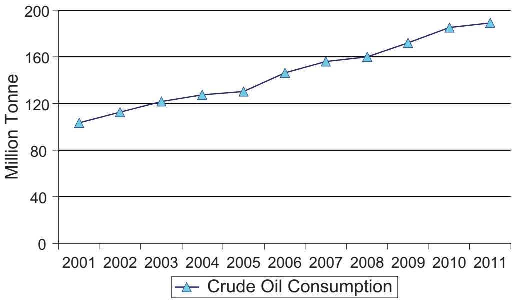

Figure 2: Crude oil consumption in India[2] with data projected beyond 2007 using a 5.5% annual growth rate.

food crops, especially in many developing countries and therefore their widespread use is considered non-sustainable and a threat to food security.[5] Many algae are known to accumulate several types of lipids to the tune of 60% by dry weight[6,7] and are now considered as a promising alternative to PDF.[8] These algal lipids are in turn potentially convertible to more usable liquid fuels—most attractive route among them is the transesterification of the triacyl glycerols (TAG) to algal biodiesel.[9] Algae, like most terrestrial plants, use CO_2 for their photosynthesis[10] and have therefore been considered as ideal candidates for CO_2 capture.[11] Algae can grow well on several types of less desirable sources of water,[12,13] including domestic and industrial wastewaters,[14,15] saline and brackish waters,[16] sea water,[17] etc., because of which they are considered as approaches that will not threaten food security of the region.

The process of development in most developing countries has come to depend strongly upon various forms of PDF, and upholds the rapid pace of development. Urban transport system has become increasingly dependent upon PDF,[1] and agricultural practices have become very highly mechanized and PDF dependent,[18] all of which require PDF in increasing quantities. This dependence is expected to rise further and remain at a high level because of a greater need for mobility.[19] Hydrocarbon based fuels are the key drivers of such a developing economy. The annual crude oil consumption has been increasing gradually from about 90 M_{toe} (1998) to about 156 M_{toe} (Figures 1 and 2). Within India, oil and gas as fuels are considered as the major drivers in national development wherein 45% of the total energy needs are met by oil and gas sources.[2]

1.2 *Sustainability-domains, manifestion and possible management with algal biofuels*

1.2.1 Land area needed, productivity estimates—advantages over non-edible oilseed crops: Cultivation of algae is considered much more remunerative in terms of oil yields compared to an oilseed crop grown on land because of many advantages the algal system possesses. Firstly, in most terrestrial crops, the crop plants have only a small part of the overall plant that is economically useful for oil extraction, namely the seed.[20] In general the oilseed forms only 20% of the total weight of the crop, e.g. groundnut. In a typical algal species the entire cell mass is harvested for oil extraction. Second, the growth period for algae between the time it is seeded till the time it achieves maximum plant density is only a week or less,[21] however, in a field crop the growth phase could be as large as 60 to 75 d. Only after this does the crop plant begin to accumulate biomass in the seeds, especially the oil. Finally, by altering some of the growth conditions, it is possible to have a year round crop of algae—this is something very difficult in most crops except sugarcane. In many algal species, there is a promise of accumulation of useful lipids up to a high value of 70%.[6,22] Thus even if one considers a moderate growth rate of 10 $g/m^2/d$, the potentially harvestable algal biomass is about 36 t/ha/yr whereas most field crop achieve a fifth to a third of such yields.

It is often stated that the cultivation of algae does not need prime agricultural land and will therefore not compete with food crops.[23] Many studies have been carried out to show that arid and desert like regions that are usually not conducive to crop cultivation can now be used for algal biofuel production.[24] Further, by the use of easily available brackish and saline waters in such regions, these water that are unfit for terrestrial crops can be used for algal cultivation and therefore will not compete with local agriculture or water needs. Thus it is possible to raise algae derived bio-fuels

(ADBF) without threats to food security. A popular illustration of this is the often cited example that in the US, using merely 0.49% of land area of US in Arizona and its brackish water, all the PDF needs of USA could be met through raising algae and converting it to ADBF.[12] Similarly, the use of marine and salt tolerant algal species makes cultivation in open seas possible without affecting land based food security issues.[25,26] In India too there is large wasteland estimated to be around 60 Mha along with saline areas, salt affected areas as well as cropped areas where a second crop is possible but not practiced. This needs to be examined in order to determine the suitable areas for cultivation of algae and production of algal biofuels as well as their sufficiency to meet current and future biofuel needs. While a lot of research effort and reviews have been brought out on the research leading to improved algal biofuel production capability in terms of various forms of photo-bioreactors as well as genomic and meta-genomic approaches to increased lipid accumulation in algal species, there is little by way of addressing the likely sustainability issues that will emerge should the world rapidly switch to algal biofuels. The key domains that will need to answer sustainability criteria are

a. The possible locations where algae will be cultivated without affecting food security of the region
b. Sustainable methods to meet various process inputs such as water, nutrients
c. Practices that ensure recycling of all nutrients so as to minimize losses and additional fossil fuel use
d. Approaches that minimize the C-footprint of the overall system and biofuel production, processing, distribution and final end-use.[6–8,12–15,21–25]

1.2.2 Plant nutrition required for algal biofuel cultivation: Just as crop plants are provided fertilizers to supplement shortages of plant nutrients in the growing environment, the soil, algae cultivation too requires supplementary plant nutrients that are unlikely to be available in the water source on which algae are being raised (with few exceptions). These nutrients are commonly represented as N, P, K as well as other macro and micro nutrients. Research reports suggest that between 0.3–0.5 kg N, 0.1 kg P and 0.1 kg K to 0.33 kg N, 0.71 and 0.58 kg P and K[27], respecitively are needed to produce 1 kg algal biocrude (lipid). When we attempt to substitute the current crude consumption in India at 150 M_{toe} crude/yr, the nutrients needed to produce algal biofuels would be 80, 16 and 16 Mt of N, P and K, respectively. Even if we consider that the efficiency of the fertilizer use would be as high as 50% in such an aquatic environment, the annual additional NPK needs at the national level would be 160, 32 and 32 Mt respectively. Under conventional understanding of additional algae cultivation this would mean an additional burden to the current levels of fertilizers provided (significantly subsidized). When we consider that today about Rs.1.2 trillion (extrapolated and adapted from GOI, 2009),[28,29] is the current level of subsidy being provided for fertilizers, the additional subsidy burden would simply be double the current levels. The fertilizer subsidy

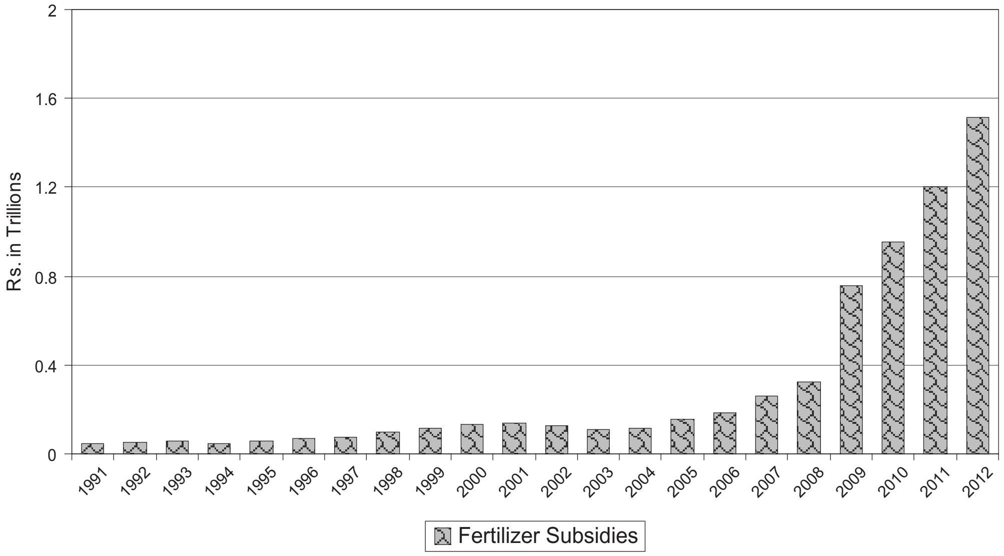

Figure 3: Fertilizer subsidies in India (GOI, 2009).

growth rate had leaped significantly (28%) during 2000's as against 13% percent during the 1990s as international prices of fertilizers and raw materials, feedstocks and intermediates have increased dramatically during the last couple of years.[30,31] Further, because N-fertilizers are manufactured from fossil fuels such as naptha or natural gas, this additional need for N and other fertilizers to raise algae for biofuels will greatly increase the already high level of fossil fuel dependence. Thus to raise algae in India, other sustainable alternatives that do not need constant use of fossil fuel dependent manufactured N needs to be found.

1.2.3 Water required for algal biofuel cultivation: Algae need to be cultivated in photobioreactors, or less complex systems such as race-ways and open ponds. All these require large quantities of water. Open surfaces of water in an arid, semi-arid or even sub-humid climatic region would lose water through evaporation losses, and the water lost needs to be replenished daily to ensure that the water levels in the cultivation pond (or similar device) remain at the required level. This means that in all algal ponds or bioreactors, water would be lost to evaporation or cooling to ensure the operating temperature is appropriate, and a certain mass of water needs to be sacrificed for algal biomass production measured as litres of water that is irretrievably lost or sacrificed for each kg of algal biomass produced (or for each kg of algal crude produced). From various reports of algal productivity and normal evaporation from open surfaces it could be surmised that generally, at an algal productivity of 10 g dry matter/m^2/d and an evaporation rate of 5–10 mm/d from open water surfaces, the water lost per kg of algal dry matter produced could be between 500–1000 L. In various warm countries such as India, the annual precipitation measured as annual rainfall in mm is much lower than the potential annual evaporation (as mm/year). Sustainability approaches then dictate that water lost (or sacrificed) as evaporation in algal cultivation should not exceed annual rainfall for that location. This in turn suggests that algal cultivation can be practiced for a period where the cumulative water loss does not exceed the total annual precipitation. Therefore, the period for which there is potential to ensure that the water lost by evaporation is less than the annual rainfall could vary between 60 d to 270 d. This component is further complicated by the fact that there is very little land area available which does not compete with crop cultivation and algae production and can be considered only after a crop is cultivated in the particular area. Sustainability considerations also dictate that algae cannot be cultivated in dry areas, and only in reasonably wet areas where water availability-either as rainfall or as artificially accessed through other means, exceeds the evaporation loss on an annual basis can be used. All these issues are discussed later on in this paper.

1.2.4 Cultivation technology and sophistication: Photobioreactor Vs Open Ponds: Cultivation systems being used for microalgae are mainly open ponds and closed photo bioreactors (PBR). Open pond cultivation systems could be natural (lakes, lagoon, and ponds) or engineered (raceway, circular). Raceway type open pond systems are primarily used for industrial scale cultivation of microalgae. They are usually built in concrete and operated at water depths of 15–20 cm.[32] The open pond cultivation systems are relatively cheaper to construct and can be constructed in non agricultural or waste lands. The cost of construction could be significantly reduced by building plastic lined compact earthen raceway ponds and such low cost ponds may be suitable for production of microalgal biomass for biofuel applications.

Economic feasibility of open outdoor raceway ponds is well established for the cultivation of extremophiles viz., *Spirulina* (high pH) and *Dunaliella* (high salinity) which are generally protected from other competing algal species and predators and all the harvested algal biomass is used as such for food/feed/nutraceutical applications. From such algal biomass high value products like β- carotene, phycocyanin, etc. can be extracted. Large scale open raceway ponds for production of *Spirulina* and *Dunaliella* are in operation in various parts of the world including India. Mass cultivation of algae in India dates back to raceway pond cultivation of *Spirulina* for food and essential metabolites.

A large number of fresh water and marine microalgae are being identified for biofuel production based on their lipid content[7] and *Botryococcus sp.* for hydrocarbon content.[6] However, the cultivation methodologies are yet to be established for their economic feasibility as cultivation of microalgae for lipid/hydrocarbon production involves various other integrated aspects such as cultivation under stress[32] to facilitate lipid/hydrocarbon enhancement, ease of harvesting, dewatering, drying, lipid/hydrocarbon extraction and utilization of spent biomass. A major challenge in operating an open pond cultivation system is the maintenance of the algal species of interest. Being open, the ponds are prone to contamination by other competing algal species which threaten the dominance of the species of interest. One such unicelluar alga competing with *Spirulina* in open outdoor ponds has been discussed.[33,34] Application of open pond systems for cultivation of other algal species, especially for fresh water species, require robust pond management protocol in order to sustain the dominance of cultivated species and protecting it against other competing algal species. One of the approaches successfully used with *Botryococcus* was to adapt the alga to salinity.[35] Nutrient manipulation in growth medium is another approach to maintain the single algal culture. Microalgae are also prone to infestation and grazing by predators like rotifers and protozoans. Infestation with algal grazers adversely affects the algal productivities and leads to loss of algal biomass and this aspect has remained largely un-addressed. These challenges although appears formidable, could be overcome by selecting robust algal strains, adaptation of the selected strains to environmental changes and developing strain specific cultivation protocols as discussed in the subsequent sections.

Closed photobioreactor systems are being used for cultivation of microalgal species for the production of high value products. Closed photobioreactors have been discussed for theoretical advantages in terms of protection from contamination, attaining higher cell culture densities and facilitating better control over physico-chemical conditions e.g., light and temperature.[36] Various designs of photobioreactors have been discussed[37,38] and tubular, flat plate, airlift tubular and bubble column reactors are the commonly used closed photobioreactor designs (Figure 4). As per the reports available in literature, the largest closed tubular photobioreactor is the 25 m^3 plant of Mera Pharmaceuticals, Hawaii[39,40] and 700 m^3 plant in Klotze, Germany.[41] *Haematococcus sp.* appears to be only microalgae currently being grown at commercial scale using closed photobioreactor. The cost of closed photobioreactos is significantly higher than open ponds and is difficult to scale up. Reports on biomass productivity of other microalgae with photobioreactor systems are largely based on laboratory studies as currently the relatively high construction and operating costs, and complexity of operation of closed PBRs limit their large-scale commercialization.[36] There are no reports available on large scale commercial production of microalgae in India using closed photobioreactors. Some of the other challenges faced with closed PBRs are temperature control, shear stress, fouling, gradients of pH, dissoloved oxygen and CO$_2$ along the tubes, and sticky growth on walls hampering the light penetration. The prevailing opinion is that closed PBRs alone may be incapable of cost effective production of microalgal biomass for biofuel production. However, they may be used in a two phase production process where contaminant free inocula for large scale open raceway ponds are generated using closed PBRs.[42,7]

Based on net energy ratio (NER) analyses of closed photobioreactors and open pond systems of microalgal production, Huesemann and Benemann[43] reported the raceway ponds to be the only economically feasible process as it showed NER of >1. A later study corroborated with these findings[43] for open raceway ponds and also reported NER of >1 for flat-plate PBRs, thus considering both the systems as economically feasible. However, the horizontal tubular photobioreactors were considered economically unviable owing to NER of <1.[44]

Mass cultivation of algae in India dates back to raceway pond based cultivation of *Spirulina* for food and essential metabolites for humans[45,46] (cyanophycean species). Studies involving the growth of the oilagenous algae *Botryococcus mahabali* in raceway ponds yeilded productivities

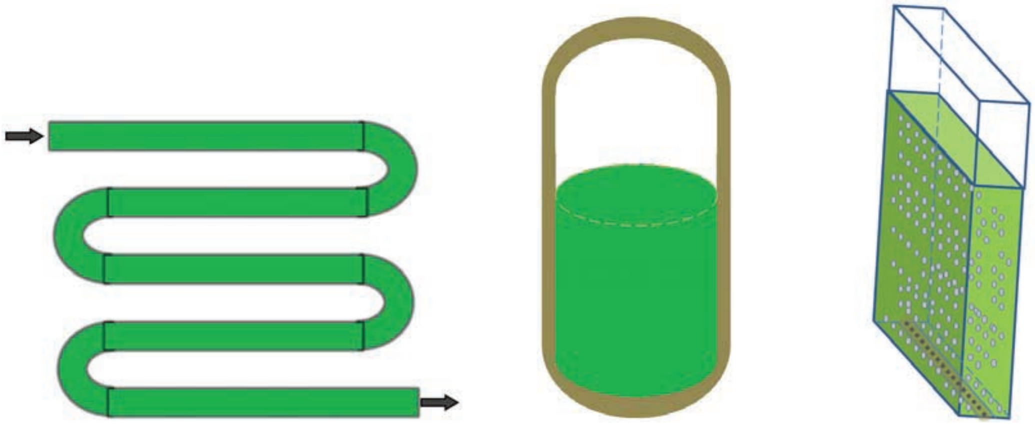

Figure 4: Schematic diagrams of three types of closed bioreactors.

of 1.0–1.5 g/L with total hydrocarbon content of 14%[47] and 9 g/m^2/d (*unpublished data*), and also provided long chain fatty acids[48] (C21-C23). In another case fat content of 22% (w/w), with palmitic and oleic as major fatty acids,[49] has been achieved. A synthetic media with 16:8 h light-dark cycle gave 33–46% hydrocarbon.[50] Optimum concentrations of mineral nutrition such as dihydrogen potassium phosphate, potassium nitrate, magnesium sulphate and ferric citrate increase biomass up to 0.65 g/L with 50.6% (w/w) hydrocarbon in 30 d.[51] Studies carried out on algal growth in wastewater treatment systems of Mysore and Bangalore reveal biomass productivities of 9.15–12.6 g/m^2/d (unpublished CST, study).

1.2.5 Centralized vs decentralized; ancillary technology needs and readiness: Considering the large requirement of biofuels, the cultivation of algae is expected to be spread over a very large cultivation area, with productivity in the range of 10–36 t/ha/yr under field conditions,[52,47] the cultivation area will be spread over millions of hectares. As discussed later, a large part of the cultivation would be potentially feasible for a period about 30–60 d in various paddy plots. Such a decentralized production also requires that the harvested algae be collected, transported and processed in a decentralized manner for extraction of algal crude, biofuel residue and nutrient rich digestate. This is because the wet algae harvested from paddy plots will usually have a low shelf life and needs to be processed immediately, and is best done at the village scale itself. Thus all the technologies related to post-harvest processing of algae is likely to be anchored in a decentralized manner—and in India at a village level. Such decentralized processing is, therefore, expected to produce a significant level of decentralized wastes in the biofuel extraction and bioresidue processing steps for which a whole lot of ancillary technologies that are capable of

a. Extracting algal crude at village level,
b. Conversion of biofuel residue to biogas at the village level and
c. Conserve recyclable nutrients recovered from processed algae and retained in a form that is either immediately, used on paddy lands or preserved till the next cropping season.

Such technologies are required to be ready and deployed before the overall algal biofuel efforts are disseminated. This approach is expected to produce a significant surplus of biomethane from the algal biofuel extraction residue. There is then a need to either use the produced biogas locally or inject it into a natural gas grid after the necessary conditioning. Such a grid does not exist today, but needs to be created soon, without which the fuel value of the gas cannot be realized in a widespread manner or appropriate remuneration achievable to the algal biofuel (biogas) producers. Much of all these strategies require that a significant part of the algal processing to be carried out in a decentralized manner and necessary infrastructure therefore needs to be evolved.

There is a likelihood that there are only a few locations in India where large cultivation ponds and centralized processing of algal crude and its extraction can be carried out to achieve an economic scale of operation. Success of algal biofuel in India will therefore depend upon a large segment of decentralized production, extraction and pre-processing of algal crude, and retaining it in a state that could be converted to immediately usable biofuels at a decentralized manner. The sustainability issues related to this is discussed in later section.

1.2.6 Potential for secondary wastes generation: The dried algal biomass can be used directly without much processing for either energy generation by direct burning or lipids can be extracted to produce algal crude, which can then be transesterified and used as biodiesel.[21] In this latter case the biomass residue left after lipid extraction, i.e., spent biomass generated is ~60%. This biomass generally comprises of the cell wall materials along with the cell constituents. This fraction can be fed to anaerobic digesters (AD) from which a large quantum of biogas may be generated on a daily basis. The digested slurry or effluent from the biogas plant that is discharged after AD is a nutrient rich liquid with very high N and P content that can then be applied to the agricultural lands (paddy) to achieve increased productivities. This in turn significantly reduces the burden of synthetic fertilizer needed to the agricultural lands. Such a form of organic nutrient addition to the crop is expected to have lower losses, reducing free nutrients being lost into the standing water in the paddy plot, that later may be lost from the system by other routes. Large extent of losses can cause eutrophication in the nearby receiving water bodies. Another promising route to utilize spent algal biomass is to ferment it to bioethanol. These secondary wastes generated can be very actively converted into utilisable feedstocks for biofuel generation and this process aids in energy co-generation that increases the adaptability of the approach to finally make the overall process much more sustainable. Such biorefinery practices would result in the rejuvenation of the zero waste culture, providing value added byproducts rather than waste—making the overall process, economically, socially and environmentally sustainable—a trait that is normally found in subsistence agricullture and traditional rural lifestyles.

1.2.7 Creating C-neutrality and negativity: The large scale use of fossil fuels to lead the development process as well as its increasing share in the development process necessitates its substitution by environmentally benign and C-neutral energy sources/fuels that do not create a net increase in GHG production. Sequestering large quantities of C can offset the increasing level of CO_2 production while allowing 'green development' processes. By sequestering additional levels of CO_2, this approach allows the continued use of fossil fuel at current rates of usage on one hand, and on the other it even allows some level of short term increase in fossil fuel energy use with an expected complete replacement by such biofuels. To achieve such a C-neutrality there is a need to increase the extent of CO_2 sequestered (in this case into algae) as well as to minimize the dependence on fossil fuel use, thereby fostering environmental as well as economic sustainability.[53,54,19,20] When a need arises among various countries to reduce their GHG emissions and C-footprints, an important method would be to reduce the share of fossil fuels with a simultaneously adopted practice of C sequestration. In such an eventuality, biofuels become an important option. When the share of biofuels in the overall mix of fuels increases, biofuels can be considered as C-neutral and sometimes even C-negative that can play a critical role in meeting the energy targets to replace PDF transportation fuels.[55] Liquid fuels used in transport sector can directly be replaced by these clean C-neutral biofuels such as algal biodiesel.[20,56–58] In recent years, there has been increased usage of liquid biofuel in the transport sector because of various policies to safeguard energy security and mitigate GHG emissions.[59] There have been many experiments and attempts to replace conventional fossil fuels in trains, buses and personal vehicles. These vehicles have been run on biodiesel as a move to be C-neutral.

2 Towards Sustainable Large-scale Cultivation of Algal Biofuels + the Indian Experience

2.1 *Laboratory cultivation and stock culture maintenance, performance deterioration*

Algal stock cultures are maintained in agar slants as well as in liquid culture in high salt strength medium at low temperature and low light to facilitate longer periods of sustained growth. Microalgal growth and metabolite production is influenced by different media constituents.[60] Prolonged culturing of an alga in a maintenance medium affects the productivity of the organisms and a separate production medium is required for large scale cultivation to obtain higher growth rate and productivities, e.g., *Haematococcus*,[61] *Spirulina* (unpublished data). Therefore it is advisable to optimize growth and production conditions separately for enhancing biomass and desired metabolite, including hydrocarbons.[51,62–65] Designing and economizing of cultivation media is an important part of development of a process for outdoor cultivation of microalgae. The designed media should impart the growth nutrients to the microalga at a lower cost and thus has to be simple. CFTRI has designed and developed one such medium for large scale cultivation of *Spirulina*, referred to as CFTRI medium.[45]

2.2 *Performance and problems of open raceway pond cultivation*

Outdoor open pond cultivation studies with microalgae have been carried out at CFTRI in circular and raceway ponds (Figures 5 and 6).


(a) (b) (c)

Figure 5: Outdoor open pond systems for microalgal cultivation (a) Circular Pond (b) Raceway Pond (c) Scale up raceway pond. Open outdoor raceway ponds have been used in many commercial production facilities in India.[70]

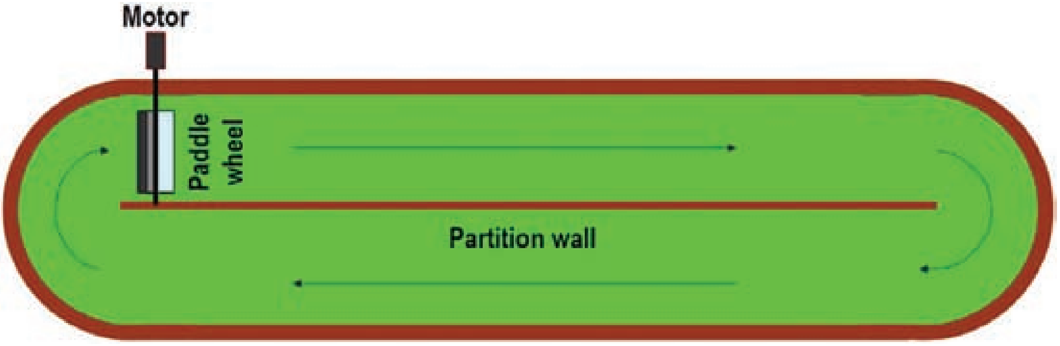

Figure 6: Schematic layout of a typical raceway pond. The salient features are i.depth 30 cm; operating depth 15 cm; Number of paddle units 1 or 2; rpm of paddles 23–36; Flow rate 50 cm/sec.

For *Scenedesmus* and *Spirulina*, biomass yields of microalgae in open raceway ponds at CFTRI, Mysore, were in the range of 15–20 g/m^2/d and 8–12 g/m^2/d, respectively.[45] In a study carried out with *Botryococcus* strain in 5000 L raceway pond, the biomass yield was 9 g/m^2/day with hydrocarbon content in the range of 15–20%. Based on this data considering 300 working days, a biomass yield of 10 tonnes could be obtained per acre with hydrocarbon yield of 1500 kg. Algal hydrocarbon does not need transesterification. Although the yield per acre are comparable with Jatropha,[64] the cost of production of biomass is higher in the case of algal biomass. In our recent trials with indigenous lipid and hydrocarbon containing algae, the yields in outdoor ponds have been in the range of 5–9 g/m^2/d (unpublished data). Therefore the selection of robust microalgae with innovations in cultivation systems is necessary to achieve higher productivities and make the process economically viable.

Microalgal cultivation in open raceway ponds is prone to seasonal variations. During summer, intense sunlight may cause damage to photosystem in the algal species leading to photoinhibition and photobleaching. Similarly during rainy season, the cell densities in pond cultures tend to get diluted whereas during peak winter season algal growth and productivity is often adversely affected due to lower temperatures and shortening of day light hours. Due to these adverse effects of seasonal variations, the selection of location of open raceway ponds is an important factor to ensure continuous production and locations with moderate climatic conditions are therefore preferred. Photo-bleaching of algae can be minimized by managing the culture depth in the pond and ponds could also be provided with cost effective sun screens, shade nets and greenhouse structure to protect the culture against high light intensity, heavy rain and heat loss during rainy and winter seasons respectively. Year long cultivation of *Botryococcus sp.* could be achieved in outdoor raceway ponds at CFTRI by taking these protective measures.[64] These solutions though effective at small to pilot scales of operation, need to be evaluated for their economic viability at large scale implementation to increase the number of days of operation in a year.

Microalgae can fix CO_2 from three different sources viz., CO_2 from atmosphere, CO_2 in discharge gases from heavy industry and CO_2 from soluble carbonates.[66] Most microalgae are able to tolerate and utilise subtantially higher levels of CO_2 than what is available in atmosphere, i.e. 300–400 ppm,[67,68] and supply of external CO_2 leads to enhancement of algal biomass production. Therefore, microalgae cultivation systems combined with external CO_2 sources such as flue gases from thermal power plants could serve as an effective CO_2 sequestration system with enhanced biomass productivities. However, devising an effective system of introduction of CO_2 to algal culture and ensuring an efficient gas transfer are important aspects and discussion on these lines are limited in the available literature. These aspects become even more critical in the case of open pond cultivation systems which are open to atmosphere and therefore prone to loss of CO_2 injected from external sources. Laboratory studies using floats have been carried out to supply CO_2 to *Haematococcus*[61,69] in the form of bubbling air mixed with CO_2 and bicarbonate to *Botryococcus*.

Although biofuel production from microalgae in outdoor ponds currently appears to be a difficult task, a steady pursual of challenges and the emerging innovations in pond design as well as efforts towards media optimization, understanding of physiology and chemistry of microalga, single and two phase algal cultivation methods for higher accumulation of lipids, improvements in harvesting techniques with recycling of nutrients, dewatering and drying of biomass and extraction methods for algal lipids and hydrocarbons, are required to establish a technically feasible and economically viable outdoor cultivation system for microalgae to produce biofuel. Further, even though there are several technical and economic challenges in scaling up and operating closed photobioreactors at a large scale in India, the tropical weather, bright and long sunshine hours along with the vast coast line, the outdoor open pond system appears to be the viable option. In many parts of India (such as parts of Tamil Nadu, Karnataka, Maharashtra, etc), a year round cultivation of alga, especially of *Spirulina*, has already been achieved and this provides a good starting point for future work in large scale production. A hybrid system combining both the photobioreactor and open pond system may also be explored—photobioreactors provide quality and pure algal cultures needed for start up inoculum for decentralized units while open pond systems provide simplicity for decentralized cultivation. This concept has been tested in a small scale. A laboratory scale study was carried out for *Haematococcus* sp grown in closed photobioreactor and then accumulation of astaxanthin was achieved in small open raceway pond.[61]

A two phase cultivation process may be required for production of lipid rich microalgal biomass as microalgae accumulate lipids under nutrient limiting and salinity stress conditions. Therefore, in first phase microalgal biomass needs to be raised rapidly under abundant nutrition using a nutrient sufficient growth medium and later the algal biomass needs to be shifted to a growth situation involving nutrient stress and C-surplus to encourage accumulation of lipids. This scheme also requires efficient harvesting method with recycling of nutrients for continuous cultivation without affecting the growth of the algal cells. Harvesting methods by auto flocculation and flocculation with natural flocculants gave encouraging results with unicellular fresh water microalgae and allowed recycling of nutrients under laboratory conditions (unpublished CST data).

Cultivation of photosynthetic microalgal biomass for biofuel purpose (under situations and locations indicated later) is considered environmental friendly and does not compete with food crops for fertile agricultural land unlike other biofuel crops. In addition, an integrated approach of using flue gas emissions with wastewater based cultivation media for algal biomass and value addition to spent biomass after recovery of lipid/biofuel is expected to make the overall process cost effective and environmentally sustainable. The decentralized nature of the proposed large-scale cultivation by various farmers as a second or multi-tier crop along with paddy is expected to provide empowerment and additional livelihoods to a large number of farmers—thereby providing economic and social components of sustainability (not discussed in the paper). The use of wastewater and industrial effluents could have growth and productivity enhancing effects on microalgae as reported for *Spirulina* grown in outdoor ponds with addition of eucalyptus Kraft black liquor, (generated in the paper manufacturing process during pulping of eucalyptus wood).[70,71] The use of domestic and industrial wastewaters for algal growth leads to an avoidance of GHG emissions, and thereby is expected to make the overall system C-negative or C-neutral and needs to be be demonstrated adequately.

3 Review of Potential and Sustainable Options for India

Sustainable technologies and definition of sustainable technologies normally refer to the triple bottom-line of environmental, social and economic components of sustainability. Thus while reviewing sustainability, needless to specify, a region, a people and an economic regime is already subsumed. Thus in order to discuss sustainability, in this case we refer to the geographical area, environmental issues and peoples of India so that the review could be reasonably precise and well grounded.

3.1 *Climatic conditions and areas suitable for sustainable algal cultivation based on water availability*

Algae are sensitive to the growth conditions including those influenced by climate and weather. While nutrient status of the water could be ‘managed’, issues such as light intensity, water temperature, evaporation loss from the algal pond surface are influenced by climate.[27] For example, although a place like Rajasthan in India could support year round cultivation of algae, the water lost in the process will be too heavy a price to pay and would be uneconomic. Suitable regions within India where algae could be cultivated without too much damage to environment or resource overuse are discussed.

3.2 *Land area needed, productivity estimates—advantages over non-edible oilseed crops*

3.2.1 Many attempts have been made earlier to show that a large scale shift to biofuels is possible by firstly cultivating woody biomass on wastelands and using secondary fuels from woody biomass for energy and/or carbon sequestration. A few of these studies indicate that out of a total land area of 328 Mha about 160 million is cultivated for various crops. About 60 Mha is not cultivated and is considered as usable wastelands.[72] However, much of these wastelands generally occur in areas which are under climate based classification considered dry and deficient in soil moisture and soil moisture holding capability. Also there are no sources of non-agricultural water, as has been visualized for other countries, e.g. US suggests the use of brackish water for cultivation of algae.[73] In most of the developing countries availability of cultivable land for alternative uses are low and such potentially cultivable lands generally have a lot of competing uses and are therefore difficult to be allocated to growing algal biofuels without concomitant threat to food security. In the case of wastelands most of such land is 'wasted' either because of extreme levels of soil erosion and neglect or because of a hostile arid climate. Such observations suggest that it is necessary to find ways by which algal cultivation can be carried out on cultivable land in the same manner and strategy as is done in multiple cropping or multi-tier cropping, and build on the previous experience of growing blue-green algae in paddy crop land.[74–77] This enables the same piece of land to be used both for crop cultivation as well as for algal biofuel production ensuring that these do not compete with each other or conflict with sustainability and sustainable development goals.

3.2.2 A single large tract of land in the Rann of Kutch area (about 3 Mha) occurs as highly salt-encrusted basin in which land is partially covered by a sparse vegetative cover (predominantly by a single bush species, *Prosopis* sp.). There is also a very low density of human population and even lower numbers of people living off the land. The area receives very low rainfall and the soil is highly saline and ground water is much more saline than the sea. As the terrain is flat and is only marginally above the sea level, this large stretch of land therefore makes an excellent low cost and non-competitive land resource on which several marine and halo-tolerant, oilagenous algal species can be cultivated. Much of this land is unfit to support common field crops and its use for algal biofuels will not pose any threat to food security. The region is however endemic to a few animals, and alternative habitats nearby or migratory corridors need to be addressed after appropriate studies. The Greater Rann and the Little Rann are occasionally flooded and they support large numbers of algal species.[78] However, there is little information about the potential of these algal species for use for algal biofuel production. Thus an area of 3 Mha may be considered as potentially available throughout the year for algal cultivation using seawater pumped from the sea nearby.

3.2.3 The high salinity found at Kutch could also be used to facilitate growing select halotoreant algae. Such extreme conditions are favourable to the cultivation of a wide variety of oilagenous algae. Many algal species are capable of growth in such extreme conditions accompanied by brackish and highly saline waters of the Kutch region in the Western India. This is a geographically unique landscape that was once an arm of the Arabian Sea and the sea bed has risen to hold on a hyper-saline ground water. Today as the land is separated from the sea by geological forces; it has become a vast, featureless plain encrusted with salt that is inundated with water during the scant rains (100–250 mm/yr). This geographical area of 3 Mha is resplendent with numerous halotolerant algal species that are generally associated with deserts or highly saline waters. Primary productivity of the salt regions inundated with saline waters show a rate of production ranging from 7.1–34.7 mg C/m^3/h in the regions of Bedi, Okha and Kutch.[79] Several micro and macroalgae have been identified in these areas. Total algal species observed in the Kutch region add up to 174, out of which 52 are green algae, 79 red algae, 40 brown algae and 3 blue-green algae (BGA).[80] The most common algae observed are *Cladophora* sp., *Enteromorpha* sp., *Ulva* sp., *Colpomenia* sp. *Dictyota* sp., *Centroceras* sp., *Cantenalla* sp. and *Compsopogon* sp.[81,82] All these observations go out to say that large scale algal cultivation is feasible and if other issues are resolved, many of the local species adapted to the area could themselves be deployed greatly reducing adaptation time if new specifes were to be tried.

3.2.4 There are few studies on the growth and productivities of the marine phytoplankton and their potential for biofuel generation under Indian conditions. Earlier studies have addressed to some extent, the C capture ability of marine planktonic microalgae as *Nannochloropsis salina* and *Isochrysis galbana* as well as macroalge *Gracilaria corticata*, *Sargassum polycystum* and *Ulva lactuca* under laboratory conditions.[83] The biomass productivities of large brown algae (e.g. *Macrocystis*, *Laminaria*, *Ecllonia*, *Sargassum*) that are commonly found near the Indian coast range from 3.3–11.3 kg/m^2/yr on a dry wt. basis averaging ~73 t/ha/yr.[84] The seaweeds upon cultivation shows promising productivities and yields for

Laminaria japonica cultivation, for example for a 7 month growing season amounted to >150 t/ha during the cultivation period.[85] Marine algal growth and productivity studies in the Hawaian Islands have showed a very high potential of macroalgal productivities in the range of 100–150 t/ha/yr when cultivated in different bioreactors.[26] In Denmark the annual production of macroalgal biomass in Fjord area was ~10 t/ha (1991). These macroalgal species were efficient in the removal of nutrients, i.e. 56% N removal (448 t) and 40% of P removal (56t) that originated from a wastewater discarded into the Fjord region during 1985 and had potentially large content of harvestable energy.[84] Similarly the Venice lagoon in Italy (50,000 ha) has been estimated to receive a substantial volume of urban wastewater, agricultural run-off and coolant water discharged from power plants—all of which are known to carry with them various loads of algal nutrients. This is reported to cause an increase in the annual bio-productivities starting from 120t (1946) to 1700t dry wt. (1985). Other estimates suggest a potential 1 million t/yr dry wt. from this lagoon.[86]

3.2.5 Commercial cultivation of macro-algae is also being carried out in India. *Kappaphacus alvarezii*, that originated in Philippines in 1960[87] is now being cultivated in Mandapam, South India.[88] It is also cultivated on a large scale in countries such as Japan, Indonesia, Tanzania, Fiji, Kiribati, Hawaii South Africa and India.[80] Today its cultivation is gradually increasingly especially around Rameshwaram and other coastal areas where *Kappaphycus alvarezii* is being conventionally grown on bamboo raft systems in calm and shallow waters of the Indian coast as shown in Figure 7. These coastal integrative approaches for algal (sea weed) (Figure 8) cultivation are meant to withstand offshore conditions and for potential expansion of seaweed cultivation to spread over the rest of coastal India. Such cultivation in areas of poor fisheries would bring in new sources of livelihoods. Designs of such growth and attachment platforms comprise of flexible elements that enable the structure to change shape to adapt to the expected wave contours. Many designs for algal attachments have been tried among them the multi triangle network systems have worked out well. The basic unit of the structure consists of a triangular bamboo unit and several such triangles can be attached to each other to form a large flexible structure that makes the design inherently scaleable. The traditional bamboo raft cultivation methods that use 3 m by 3 m rafts yield ~250 kg of fresh weight of seaweed in 45 days (Figure 9). Assuming an initial seed mass as 60 kg seaweed and considering 6 harvests in an year, each 9 m^2 raft yields ~125 kg of fresh wt./m^2/yr (@7%TS; Vadassery, Per. Comm.). Many experiments on cultivation of *K alvarezi* in tidal pools were carried out at Okha during 1994–95,[89] and attempts to cultivate it in the open sea at three localities viz. Mithapur, Okha and Beyt Dwarka and were successful.[80]

Saltwater cultivation of macro-algae has generally been for the production of select commercial products such as carrageenan etc., that fetch a high price.[90,91] These are also reported to have a significant extent of useful fatty acids.[92]

3.2.6 A large part of the shallow seas around the coast line are potential areas for cultivation of the larger macro-algae. Many other areas along the coastline form a rich fishery and are therefore not acceptable for algal cultivation. Efforts therefore

Figure 7: The cultivation of *Kappaphycus alvarezii in traditional floating raft systems*.

Figure 8: Marine algae from east coast of India.

Figure 9: Algal harvest of *K. alvarezii*.

need to be made to delineate these regions and demarcate those suitable for algal cultivation. Moreover, due to over-fishing as well as other causes, fish catch in many areas of the east coast of India is very low and is no longer economic to fish, and many fisherfolk are turning to algal cultivation and have become quite adapted to cultivation of macro-algae. Although macro-algae such as *Sargassum* etc. do not have high lipid content, they could be fermented to biogas (energy) and many useful byproducts. Biogas could be produced from the economically unimportant parts of the macro-algae and nutrients returned to the farmed area to ensure sustainability. The potential area available for this activity is not clearly estimated, however, assuming a km extent from a 4000 km coastline and 75% accessibility, this works out to about 3 Mha (this area however, needs to be accurately determined considering several competing uses and that fishing is involved).

Table 1: Region-wise potential for flooded paddy and simultaneous algal cultivation under flooded paddy conditions.

Region/State	Total geographic area under paddy (Mha)	Continuously flooded paddy (Mha)
Eastern Region	18.583	5.694
Southern Region	7.987	5.070
Northern Region	8.277	3.991
Western Region	7.385	1.742
Total (Mha)	42.232	16.497

3.2.7 Another type of land quite suitable for algae cultivation are the constantly flooded paddy lands where even after the cultivation of a paddy crop there is a 30–60 day window with adequate water for cultivation of algae. India has a very large area of land under flooded paddy cultivation. Of about 42 Mha of land under paddy cultivation, nearly half of it is under flooded irrigation method of cultivating paddy crop (Table 1). In much of the flooded paddy cultivation, the land is flooded well before the paddy crop is planted, and also from the time of transplanting the paddy seedling to about the 45–60th day (age of crop, generally panicle initiation) the vegetation is sparse and much of the water surface is sufficiently open to sky—i.e. the leaf area covering the land area (leaf area index, LAI) is still quite low as seedlings are still small. It is well known that up to the 60th day, light interception by the paddy plant gradually decreases and the level of shading can reach a level ot about 98% on the 60th day—even at this level of light interception various algae grow (discussed later).[93] Thus, as a lot of usable incident sunlight that falls on water surface, it is potentially possible to grow various species of algae during the early part of the paddy crop and rapidly harvest them for local extraction of algal crude, biogas and recycling of nutrients back to the soil—possibly the current paddy crop. In this way, algae can be cultivated without interfering with the crop growth and the same piece of paddy land could be used for two crops simultaneously, algae and paddy without interference to the main crop, namely, paddy. In low land areas of paddy cultivation, in lands that receive runoff from paddy lands upstream, algal growth is often very high. These algae are often considered 'weeds' that 'eat' into the nutrition provided for the crop plant [e.g. delta area of Kaveri or Krishna rivers in the east coast].[9] Thus, under such circumstances when algae is cultivated only for the first 60 days of crop life, there is a potential for nearly 16–18 Mha of algae cultivation area wherein cultivation is possible for 2 months (Figure 10, Table 1). This period of 60 d algal growth on 18 Mha could, for purposes of calculation, be equated to about 3.6 Mha equivalent of year round cultivation of algae.

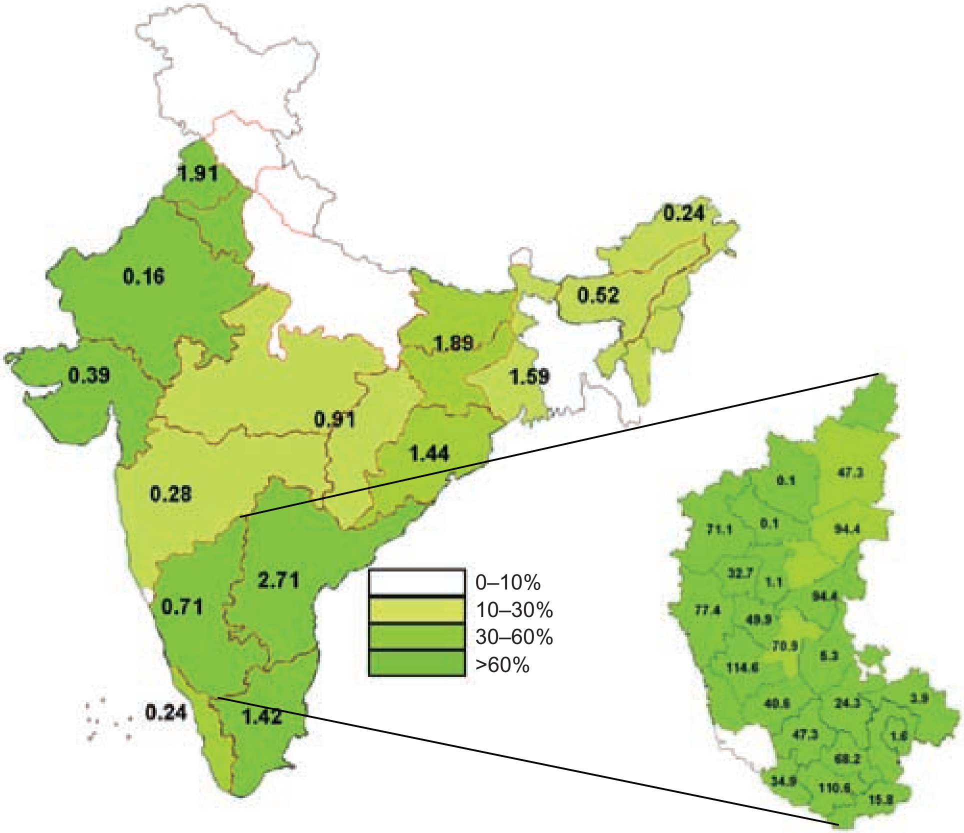

Figure 10: State-wise distribution of irrigated (flooded) paddy in India (in Mha, insertions). The inset map of the State of Karnataka shows the district-wise irrigated area under flooded paddy conditions as well as the dominant mode of paddy cultivation. The numerals inserted in the districts indicate '000 ha of land under flooded paddy cultivation in each of the districts. A large part South and Eastern India is suitable for algal cultivation.

3.3 *Algal nutrition required for algal biofuel cultivation*

3.3.1 Large scale algae cultivation, as indicated in the earlier section, would need large quantities of plant nutrients NPK, macro and micro nutrients. When cultivated along with paddy, both algae and the paddy crop will need nutrients. The extent of nutrients that would be required is discussed under section 1.2.2 and uptake of nutrients by algae in open ponds is discussed in a subsequent section on wastewater. The additional nutrients to be provided for algal cultivation will increase the need to manufacture N. Since much of the nitrogen manufactured today is from fossil fuels, the trend will increase the dependence on fossil fuels. Thus, unless efficient methods are devised that allow complete recycling of nutrients from harvested algae to the paddy crop, as discussed later, the system would be unsustainable. Furthermore, as discussed earlier, algal cultivation and subsequent biofuel extraction would require between 0.3 and 0.5 kgN/kg biofuel produced. Extending this to a country level demand, in order to generate the required level of 150 Mt_{oe} of algal crude, there is a need for 150 Mt N to be added assuming that only 50% efficiency of N is transferred from applied fertilizer to algal biomass. Although a somewhat higher N-uptake efficiency of 50% is assumed for algal biomass returned to paddy soil under flooded conditions, it may be expected that since N will be applied on a daily basis, there will be a better control on denitrification and volatilization losses and might reflect the situation in the field. Under normal paddy cultivation conditions the N uptake by paddy crop could be as low as 30% due to various modes of N.[95] Secondly, many algal species can utilize ammonia-N directly from the water as the N-source and hence volatilization losses are expected to be low, and finally when digested algae is returned back to soil after biogas production, this is expected to function like a slow release fertilizer and losses could be reduced further. Thus using only one time input of fertilizers, two crops could be raised either in the same season or the following season. Further, strain selection process would also incorporate such traits as fertilizer use efficiency to ensure that overall N losses would be much less than, 50–80%. Currently there is little research information that can predict the overall N losses and therefore further studies are required. A similar set of issues are required for P and K that will become constraining under such situations. At present little is known about overall efficiency possible when N is applied for dual purpose of fertilizing algae as well as paddy simultaneously, and all N captured by algae is recycled back to the paddy land for use by the crop in the same season or the next.

3.3.2 In the case of algal ponds in marine and halophilic ecosystems such as sea shelf and Kutch area, finding alternatives for recurring use of micro, minor and major nutrients requires that harvested algae without significant losses is initially processed locally in a decentralized manner such that the nutrients within algae can be recycled back to the area where algae was raised. Today the commonly known method to convert the residual material left after extraction of algal-crude is biomethanation. There have been a number studies on biomethanation and energy conversion and have been thoroughly reviewed for marine macroalgae *Macrocystis pyrifera* (giant Kelps), *Sargassum* sp., *Laminaria* sp., *Ulva* sp., *Gracilaria* sp. etc as potential sources of methane.[96] The marine kelps have very high growth rates and added to that they are easily biodegradable because of absence of refractory lignocellulosic compounds that are typical of terrestrial biomass forms.[97] Marine species like the giant kelp have a methane yield of 103–310 ml of methane/g of dried algal solids.[97,98] The anaerobically digested algae, like most biogas plant digestates, are expected to be a dark and turbid liquid rich in organic N. Techniques that return these nutrients back to the standing algal crop without concomitant increase in turbidity, interference in light availability, bacterial growth etc. needs to be developed.

3.3.3 In the case of simultaneous cultivation of paddy and algae, the strategies adopted for raising mixed algal species with a blue green algae component could be carried out here as well. Earlier studies on raising blue green algae in paddy fields have shown that in algae fertilized paddy fields initially there is a lot of green algae (>90%) which slowly shift to about 30–40% blue-green algae (BGA) to achieve between 1–6 t of algal biomass/crop cycle and add a net increment of about 30 kgN.[99] There is a lot of experience in this area and research results suggest that nutrients meant for the main paddy crop could first be applied to fertilize the algal crop, and since most of the algal biomass would be incorporated to soil during the existing crop cycle and the decomposing algae would in turn provide the required nutrients to the standing crop, only a minimal fertilizer needs to be applied to the main paddy crop. This is because nutrients picked up by the blue-green algae that is harvested is recycled back to the paddy plot. In a similar manner, algae raised for biofuel that are harvested during

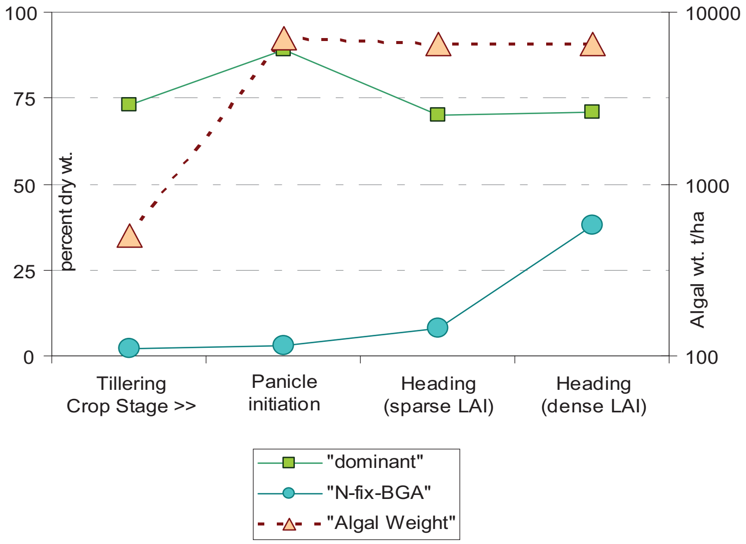

Figure 11: The increase in algal biomass (dry weight basis), dominant species and BGA levels at various stages of paddy cultivation (adapted from Roger and Reynaud[75]).

the crop cycle would be processed simultaneously for extraction of algal biofuel and the process residue (after extraction of algal crude and biogas) could rapidly be returned to the soil. Such a nutrient management strategy had been successfully adopted for cultivation of BGA as well as *Azolla* in the past.[99–101] This would require algal harvesting systems in the field as well as rapid algae processing systems to function in the immediate vicinity, and therefore calls for decentralized processing.

3.3.4 Several blue-green algae and green algae have been cultivated in paddy fields in the past with the objective of increasing the net N content of the system.[102] It has been reported that up to 40 kg N/ha could be fixed by the simultaneous cultivation of these algal species and *Azolla*.[101] One of the most successful strategy was to provide the algae-*Azolla* combination (*Azolla-Anabena*) with all the P and a small quantity of N at the time when these symbionts were inoculated into the paddy crop land. *Azolla* (and the blue green algae symbiont) grew till about 60 d of crop life after which they became shaded. These were then trampled and pushed back into the soil where they decomposed rapidly to provide nutrients to the reigning paddy crop (this is discussed in another article in this issue). The key strategy was that the P and K as well as some of start-up N needed for the paddy crop was provided to the *Azolla* crop instead. Later, when *Azolla* was trampled and pushed back into soil around 30–60 day of the paddy crop, it released the 'locked' nutrients to the paddy crop and significantly increased the overall N of the soil system. This strategy reduced the N needed for the overall system because the moderately decomposing *Azolla* ensured that most of the released nutrients were picked up efficiently by the paddy crop in the very same way as slow release fertilizers would function. This also reduced the potential losses that would have arisen from the direct use of urea[95] (up to 75% N lost) and such losses are best studied using an eco-system approach.[103] The very same strategy and practices now need to be adopted to raise algae for biofuels on paddy lands such that only a marginal level of additional nutrients need to be provided for raising algae and recycling, and capture by main crop is expected to achieve a high level of N capture efficiency.

3.4 *Water required for algal biofuel cultivation*

3.4.1 One of the simplest methods of algal cultivation for biofuels would be to raise algae in open water ponds—on the sea or land (as indicated earlier). However, a major drawback of open water surfaces is that open water surfaces lose large quantities of water through evaporation (measured as mm/d). This could range between 5–10 mm or sometimes more (in rainy or winter and summer months respectively).[104] Large geographical areas of India generally have a water deficit budget wherein the total evaporation measured on an annual basis usually exceeds the total annual precipitation—suggesting the presence of varying degrees of aridity. Second, as most of the useful and nearly flat lands are already cultivated, a potential option to cultivate algae on the same farmed land can be realized only after a crop is grown or without sacrificing the outputs of the said crop. Even if there is a hope for surplus water in the region, raising a food crop needs to be given priority to ensure that food security is maintained. In this way algal cultivation need not be raised by replacing a food crop and thereby threaten the food security of the region. Using this approach to determine the presence of surplus water, various parts of the country could be classified into zones where there exist more than 150 d of acceptable water balance (90–120 d crop + 30–60 d algal cultivation), 150–210 d (30–60 d algae), 210–270 (two agricultural crops or one field crop + 90 d algae cultivation) and finally locations with >270 d of positive water balance (Figure 12). This shows potential areas where there is a likelihood of surplus of water available for algal biofuel production after cultivating a normal field crop. In this analysis, as the north east and the northern Himalayan regions are mountainous and large tracts of flat land are difficult to find, they are not included in the projection for algal biofuel production. For the rest of the country, only a small part of the geographical area—the dark hatched area and the dark blue areas have the potential climate to cultivate algae in the open using natural water sources that may be collected in the area. In this projection an attempt is made to show that there is potential to cultivate algae in these zones using a simple approach, however, a detailed approach using larger needs of water needs to be taken into account for more precise local estimations. The added water burden emerging from evaporation of water from open water surfaces such as algal ponds will not lead to major water deficit conditions and therefore it makes the overall process sustainable from this perspective.

3.4.2 Algal productivity under field conditions has been reported to range from 5–15 g dry matter/m^2/d[105,106] (avg.10 g/m^2/d). Further, as indicated earlier, the evaporation loss from algal

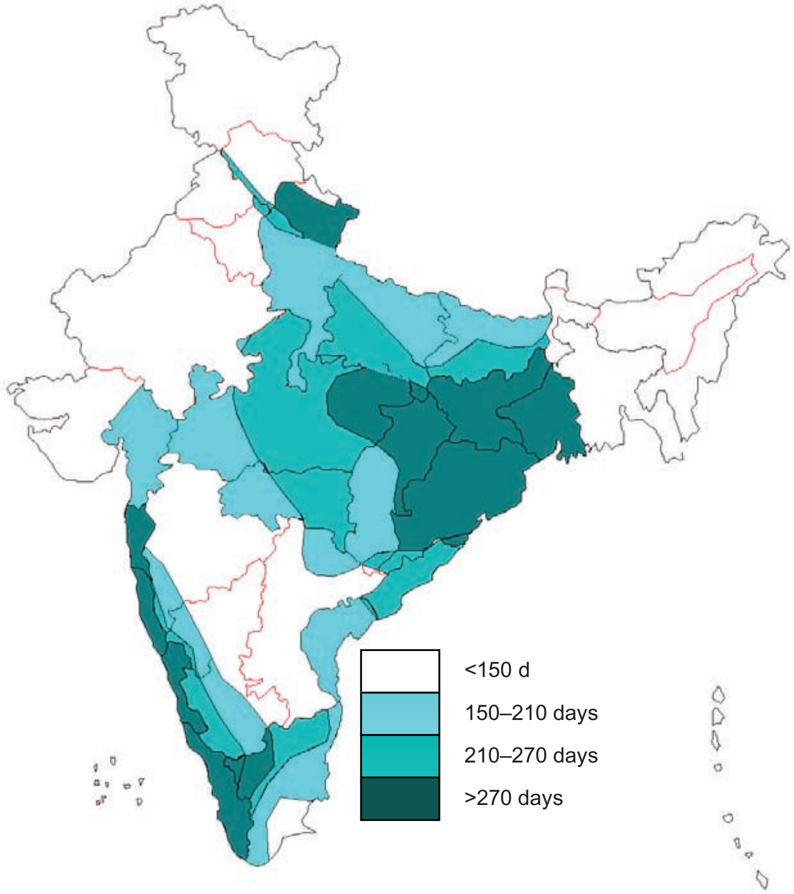

Figure 12: Wet regions of India where algal cultivation is technically feasible measured in terms of adequate availability/supply of water after meeting the needs of a single or double crop. This is determined as a function of the period for which the cumulative open pan evaporation (OPE) is less than the total annual precipitation measured in days.

ponds could be in the range of 5–10 mm/d. This then suggests that in rainy and winter seasons, when evaporation losses are around 5 mm/d, the water requirement would be around 500 L/kg dry matter and at 20% lipid content it would mean about 2500 L/kg algal crude produced. It has been reported that the water requirement for producing 1 kg of biofuel (biodiesel) is around 3726 kg with no recirculation, out of which around 15% is lost by evaporation etc. and the rest is discharged, and if recirculation loop is applied the water input reduces by 84.1% but such systems might be inefficeint to cater to the daily loads of water influx.[27] When photobioreactors are employed although the water losses are low, the need to manage heat requires sacrificing substantial water to cool largescale bioreactors exposed to sunlight to radiate out 5–7 kWh of heat. Studies on generation of bioethanol estimate a requirement of 4000 L of water required for the generation per litre of ethanol.[107]

Similarly, when cultivated in summer, there is generally a promise to achieve a higher yield from higher light intensity and longer daylight. However, assuming the earlier productivity along with higher evaporation losses, the water requirement would be 1000 L water lost/kg algae or 5000 L water/kg algal crude produced. Both these levels of algal dry matter production are far lesser than what is possible in field crops in relation to fresh water. In this context therefore it is, water-wise speaking, not economic to cultivate algae as a single crop with fresh water unless, the water used for algal cultivation is not suitable for field crops. On farm land therefore, it does not make 'resource sustainability' sense to cultivate only algae as a single crop even if the land is wasteland because there are other profitable and sustainable options for efficient use of water for cultivation of field or tree crops at higher water use efficiency (WUE). In areas affected by salt or salinity, the use of salt or sea water is of lesser consequence even though the evaporation loss is high because these types of water sources are not suitable for field crops, there is plenty of such water but no suitable economic option with higher economic gains in the vicinity. Thus from the water loss perspective it could be surmised that when using fresh water as the primary water source to raise algae in open land, the low water use efficiency of algal cultivation dictates that any other crop would be more economic and efficient compared to algae in semi-arid and sub-humid conditions of climate (such as India). Algal cultivation for biofuels could become a better option only when using sea water or when co-cultivated with paddy under flooded conditions (similar to multi-tier cropping or raising BGA or *Azolla*).[101] The potential for multi-tier paddy-algae cropping is discussed later on in this paper.

In the presence of a standing crop such as paddy, the water lost from a standing crop is generally lower than what would occur from an open surface for typical field crops. As there are insufficient generalization or experimentation about the heat absorbed by the standing water under paddy crops and therefore the extent of evaporation that is likely to occur both in still air and/or typical windy situations during crop cultivation, data needs to be generated as to what is the expected water loss from a paddy field when algae are cultivated in the water below under field conditions. The regions where cultivating algae would be sustainable in terms of careful use of water could be estimated in terms of whether there would be a negative water balance in the region. Thus in a somewhat dry region such as India, sustainability dictates that algae needs to be grown only up to a period where the cumulative annual evaporation (evapo-transpiration) does not exceed the average annual rainfall and after meeting all local demands for fresh water. Since not all parts of India receive high levels of rainfall, the potential regions of algal cultivation for biofuel will also vary accordingly and is discussed later on (Figure 12). Scenarios that use judicial combinations of total evaporation and potential evapotranspiration is likely to fine tune the estimation and needs to be done at a local level to determine feasibility and sustainability of water used for algal biofuels.

3.5 *Centralized vs decentralized: ancillary technology needs and readiness*

The large expanse of algal cultivation possible in Kutch enables the deployment of large algae processing systems (deploying economies of scale) as well as permits extraction of a large number of saleable components from the algal biofuel project such as pigments, proteins, metabolites etc.[76] that are not economic at village scale operation or requires a higher level of skills that is not affordable in decentralized village level processing systems. Similarly, oil extraction from algae has many options some of which are efficient in a large scale only. However, carrying wet harvested algae or the need to dry them, package them and transport them to centralized processing places, necessitates sacrificing a part of the algal biofuel into the transporting needs of the overall production process. This will increase the C-footprint and needs to be minimized by evolving and adopting decentralized techniques to extract and process algal biofuels at a village or cluster of village scales of operations. Both types of technologies will be needed. A detailed discussion of techniques for oil extraction is not taken up in this review—but can be found elsewhere.[22,108–110]

3.5.1 Local and decentralized processing and generation of secondary wastes: Processing of algae to several by-products in a centralized or decentralized manner is expected to produce a lot of wastes, spillages, rejects, etc. There is a need to determine the environmental impacts of these as well as environmental footprint of the wastes if not managed well. Consequently, there is a need to evolve local uses for these ‘wastes’ such that there are no environmentally sensitive by-products generated, and all wastes are re-used locally and ploughed back into the algal production processes. As the processes of lipid extraction or recovery of secondary algal metabolites are expected to differ as per the scale of operation as well as the extent of by-products extracted, there is a need to evolve and adopt ‘zero waste’ types of decentralized processes. Without such approaches a lot of non-point and decentralized sources of water and soil pollution may be expected such as those that occur in the processing of several agro-processes, e.g coffee-pulping,[111] rubber sheet making,[112] desiccated coconut production (CST, unpublished study), sago making from rice,[113] and air pollution (rice puffing, jaggery making, etc).

3.6 *Potential for secondary wastes utilization*

Although a lot of wastes and spillages of algae and by-products are likely to occur in the decentralized processing and may not receive adequate attention or regulation, most of these are expected to be quite biodegradable and will gradually decompose on the soil where stored or spilled. However, their inadvertent transport by run-off into nearby water bodies can cause adverse effects of eutrophication, nitrate accumulation etc. On the other hand these would be resources wasted and needs to be brought back into the production process. One approach, as carried out in coffee pulping process[111] and sago industry in South India, is to convert the wastes and wastewaters into biogas through anaerobic digestion that is required for the upstream processing of agro products. Quite often the poor waste management would be the only source of GHG emissions and causes convertion of the overall production process to become completely green[114] (ICWD) as well as acquires negative C-footprint and sustainability. Such approaches are necessary to ensure multiple levels of sustainability and C-neutrality even as these processes are disseminated in a large way. When, the wastewater is treated anaerobically to recover methane for diesel substitution and the treated wastewater is recycled on land, the coffee processing becomes completely green.[111]

3.7 *Energy efficiency and carbon neutrality/negativity*

3.7.1 Research carried out with a broad purpose of sequestering large quantities of C (as microalgae that settle to the bottom of the ocean) by Fe and nutrient fertilization at the sea surface has also triggered research on the possibilities to use micro and macro algae in various shallow seas as well as for algal biofuels.[115] Linking algal cultivation for biofuels with C-capture appears to provide a greater promise today. Firstly, sequestering C generated from nearby coal fired power plants provide both a low cost source of CO_2 as well as some degree of the cultivation cost is offset by the C-sequestering process being carried out for the power plant.[116] This provides algal biomass at much lower costs than that would be by cultivation algae for biofuel alone. Further, the promise of improved strains and increased lipid accumulation emerges from various biotechnological research around the world and these make it much more attractive.[6,8,20,117,118] Towards this direction, much of the research here has been focused on screening oil rich algae and finding suitable growth conditions for greater oil yields.[119,120] There has been a very large level of studies for scrutinising bio-chemical environments that triggers neutral lipid accumulation (TAG's) in algal cells for example nutrient stress as N and P limitation[7,121–123] and Fe limitation[124] etc. One of the major limitations of such type of approaches is decreased biomass productivities of cells. So, even at higher lipid yield the lipid productivities are low.[120] Therefore it is suggested to have higher biomass yield than accumulating lipid in algal cells by inducing stress conditions. Higher biomass yield can pave ways in an economic route of energy generation through other biofuel by using different bio-chemical processes. All these provide for greater hopes and opportunities to expect that the oil crisis could be mitigated through algal biofuels across the world and algae have various advantages that make them better candidates as compared to the terrestrial biofuel/bioenergy options.

3.7.2 Further, considering that the production of every kg N manufactured requires nearly 2 kg of naptha or equivalent in current manufacturing processes, cultivation of 150 Mt_{oe} algal crude would require 300 Mt_{oe} of energy to make the N required for growing it. Clearly, therefore alternative mechanisms to provide N for algal cultivation need to be devised that do not require primary use of naptha or their equivalent for manufacture. Novel methods and practices for nutrient recycling and management accompanied with low losses will then be required.

3.8 *Multi-tier cropping of algae with paddy*

Paddy cultivation is often carried out by flooding the land prior to the crop as well as during the cultivation of the paddy crop. As India has a very large area under paddy cultivation, there is potential to simultaneously cultivate algae and paddy. In fact because the land is kept flooded for a significant period, algal cultivation becomes possible because the crop cycle for algae could be as short as 7 d and many such crops can be taken up before the paddy crop is transplanted as well as raised simultaneously with the paddy crop during the early stages of the crop when the leaf area of paddy crop is low and significant extent of light reaches the water surface (Figure 13). Many issues then dictate this possibility namely,

a. prevention of competition between paddy and algae for nutrients and novel nutrient management
b. sufficiency of light for algal species deployed under various paddy cultivation conditions
c. ability to constantly recover algae rich water, harvest algae and return the water back to the paddy plot
d. ability to process algae locally so as to separate the algal crude, algal biomass and finally evolve

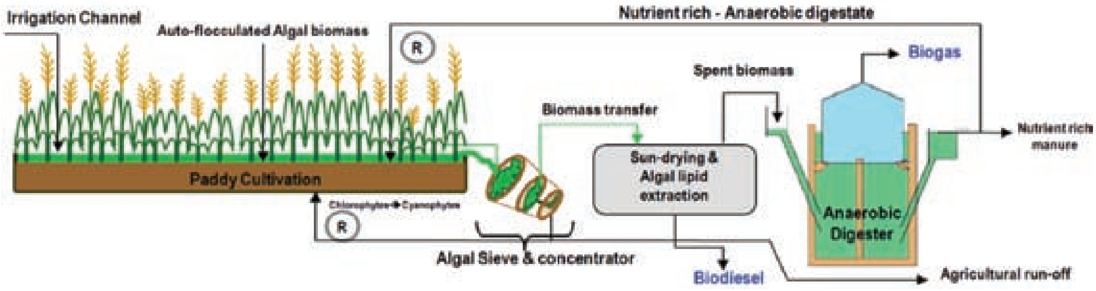

Figure 13: Schematic diagram of Algal cultivation and biofuel production in Paddy fields.

a mechanism to return the nutrients locked within algae back in time for the crop and

e. evolve algal cultivation practices that avoid build up of grazers and other species that consume algae and lower algal yields

3.8.1 Potential cultivation area for algal biofuel production: Paddy in India is usually cultivated where water availability is high either from a high rainfall pattern or with water brought in to the area by various types of irrigation systems. Cultivation practices vary significantly between regions, areas and even within villages, and therefore it is difficult to generalize for the entire country as to what will be the depth of water that will be maintained in the paddy plot and the duration for which it will be maintained among the seasonally flooded and constantly flooded types of paddy cultivation. This varies significantly even within a village. Yet, three to four types of cultivation practices are generalizable—rainfed upland, rainfed with partial flooding, irrigated with constant flooding and deep water paddy. Only the last two types have the necessary certainty of water availability as well as a sensible potential to raise a simultaneous algal crop along with the paddy crop. Further, only irrigated paddy with constant flooding makes current economic sense to raise a simultaneous algal crop because it is possible to raise algae for about 30–60 d giving at least 4–8 simple cycles of algal harvest (batches at 7 d intervals) although under continuous culture the yields could be higher. For this purpose various states of India could be classified into zones, its states and districts (illustrated for Karnataka) where the area of flooded irrigation is practiced for paddy cultivation (Figure 10 and Table 1). The total cultivated area is also highlighted. This approach suggests, as mentioned earlier, an area between 16–18 Mha is potentially available for simultaneous cultivation of paddy and algae. In order to simplify and provide a uniform estimate the following assumption can be made. In this 18 Mha of land, considering a minimum of 60 d is possible for algal cultivation, the land area equivalent 18 Mha for a year round (300 d) cultivation of algae could be equated to about 3.6 Mha of continuous cultivation.

3.8.2 Redox potential management and methane emission control: Algae cultivation in flooded paddy plots is expected to provide another set of indirect benefits for the environment and climate change process. The cultivation of a high density of photosynthetically active algae is expected to greatly increase the oxygen content of the water flooding the paddy field[125–127] and even reach super-saturation levels of dissolved oxygen reaching upto 20 mg/l. High levels of oxygen for a greater part of the day, especially in the boundary layers between the soil surface and liquid layer[128] is expected to drive the system to become oxic and therefore inhibit methanogens. Methanogenic inhibition in paddy plots is therefore expected to reduce the methane emissions from paddy lands and therefore be environmentally beneficial. This phenomenon has, however, not been proven in the field and needs to be conclusively studied/determined that high intensity algal cultivation in a flooded paddy field at required algal density will avoid or overcome methane emissions normally emitted from paddy fields—a major source of GHG in developing world. This approach, on the one hand, side will provide GHG free biofuel, and on the other side avoid methane emissions from flooded paddies—a double benefit.

3.8.3 Possible areas of crop-algae conflict: By growing algae on paddy plots, the cropping intensity would be greatly increased, therefore increasing the complexity of agriculture being practiced.

a. For efficient realization and recovery of the algal biofuel, it is imperative that the algal growth achieved is rapidly harvested at the field level.

Paddy farmers need to adapt to such a higher level of farm and resource management.

b. Wet harvested algae are expected to have a short life and possess between 90–95% water that needs to be firstly dried to ensure better recovery of algal lipids. Typically this would be the domain of the farmer. This then requires a drying mechanism for drying the harvested algae—drying algae during typical monsoon season is expected to be difficult and energy efficient alternatives need to be devised.
c. Harvested algae need to be rapidly processed for bio-fuel extraction. Many of the algae—especially BGA autolyse rapidly after harvest and therefore need to be processed for bio-fuel extraction, protein and carbohydrate recovery and finally energy recovery so that the material can be returned to the paddy growing plot to ensure its fertilization and higher yields.
d. The byproducts of the biofuel extraction process (defatted algal biomass) are expected to be about 4 times in weight compared to the algal biofuel recovery. Under such a situation, the oil-extracted residue needs to be converted to biogas and the process of biomethanation needs to be adapted to remove appreciable levels of decomposable C. In this approach to fossil-fuel substitution the decentralized processing reduces the overall C-footprint arising from land based transportation of raw materials but requires infrastructure to be created to collect and distribute the semi-finished fuels. The overall process requires close supervision.
e. The need for rapid conversion of algal biomass to biogas and other locally useful sources of energy is expected to drive the system forwards towards higher sustainability. However, this requires the active participation of the farmers—who are expected to benefit from the sale of bioenergy. There is a learning period for farmers to accept and use the higher benefits through better management of locally generated energy and fuels.
f. The digested residue from anaerobic digestion of algae is expected to be recycled back to the paddy plots. However, this requires rapid use and recycling of these organo-fertilizer precursors. There is not enough data to convince that the efficiencies and cost recovery is the highest for this option.
g. Thus many forms of local processing are expected to produce a large extent of not-so-desirable outputs from the energy route. These need to be harnessed locally and required infrastructure needs to be in place.

3.9 *Recycling and reuse of wastewater for algal biofuel production*

3.9.1 Wastewater generation and algal growth: Large volumes of wastewater are generated in cities of developing countries cities and are often let into the nearby environment without adequate treatment steps. These wastewaters are rich in plant nutrients such as N, P, K and other minor nutrients. Today, all the fertilizers used in agriculture (especially N based ones) are manufactured, transported and supplied at the expense of fossil fuels—petroleum derived or natural gas, and therefore have a large C-footprint besides becoming scarce. Much of current algal biofuel research envisages growing algae on such wastewaters. Using wastewater as the primary feedstock firstly helps to generate substantial quantities of algal biomass as alternative energy sources. At the same time they potentially recover, recycle and remove high energy containing N from wastewaters. When grown for algae, these wastewaters are treated without simultaneous emissions of methane, a potent greenhouse gas that is normally emitted during anaerobic-aerobic treatment of wastewaters, thereby avoiding the GHG emissions from the normal water treatment steps and greatly reducing the C-footprint. There is thus an increasing need for technologies and strategies that help in generation of renewable energy from algal cultivation so as to make algal biofuels C-neutral.[4,129] As the extent of usable water is generally in short supply and therefore has become scarce in a large part of the developing world, especially in India, algal cultivation in wastewater systems also meets the wastewater purification requirement without inputs of fossil fuel derived energy, high capital and operating costs, can become aesthetically acceptable and simultaneously bring back wastewaters into a reusable form. Much of the foundation of sustainability is built on the principle of reuse of water, recovery of potentially polluting nutrients, removal of potentially toxic and undesirable elements and recycling of useful nutrients into the food production system etc. Today there is little understanding about various challenges to sustainability that are likely to occur under large scale cultivation of algae for simultaneous algal biofuel production and wastewater treatment and therefore needs to be reviewed, assessed and quantified.

Domestic and municipal wastewaters are inexpensive and effective algal media for algal growth and subsequent harvest and recovery of algal-products (biomass and biofuels). Microalgae are the key players in treatment of domestic wastewaters in units such as oxidation ponds or oxidation ditches.

The growth and function of algae in such systems are generally left to natural processes and are poorly controlled. Algae have also been deployed in low-cost and environmentally friendly wastewater treatment compared to other more commonly used energy intensive treatment processes.[130–135] However, even in such cases, the processes deployed while being highly dependent upon algal growth and function, algal biomass is rarely harvested or utilized in an economic fashion.

In many developing country situations although large quantitites of wastewater is generated the technologies that have been deployed to treat the wastewater has gradually become expensive with biological (aerobic), and chemical based systems.[136] The N and P concentrations of municipal wastewaters of large metros of India range from 30–100 ppm and 10–45 respectively;[137–139] over 1000 ppm in agricultural wastewaters[130,140–142] or swine/piggery wastewaters.[142] In India, in the past agricultural wastewaters rich in nutrients were almost always reused on farm land enabling nutrient recycling and recovery. However, today agricultural practices have become mechanized, market centric and labour cost sensitive, therefore smaller proportions of agricultural wastewater are produced and recycled on the farms and significant proportions of such wastewaters are being discharged, e.g. coffee pulping wastewater.[109,112] Algae being rapid assimilators of nutrients, effectively grow in these nutrient rich environments and therefore it makes them the most suitable contenders for sustainable and low-cost wastewater treatment[8,14,20,143–145] with simultaneous algal biofuel generation potential.[21,146]

According to recent reports of CPCB the wastewater generation in the country is around 40 billion litres per day mostly from urban areas and from that barely 20–30% of the generated wastewater is subject to treatment. On the one hand, per capita water supply in India is 189 liter per day,[147] whereas production of sewage is estimated to be 138 liter per person per day. This indicates the magnitude of the problem. When we account for all losses, around 80 lpcd reaches the receiving water-bodies. In Figure 14 an attempt is made to show the volume of the wastewater generated by the cities of various categories, the treatment facility available and the fraction of the wastewater treated.

3.9.2 Nutrient assimilation from wastewaters: The nature and the degree of variability of the present day wastewaters generated is very high. Even in such wastewaters different types of algal communities thrive by their ability to utilise abundant organic carbon and inorganic N and P in the wastewater and bio-accumulate certain toxic elements as heavy metals and xenobiotic compounds.[14] The use of algae in wastewater treatment is not new and has been known from 1950s.[130] However, the chemical and biological (generally with surface aerators) processing of wastewater effluent and the resultant activated sludge are still practised world-wide as the conventional treatment method. The global level utilization of the algae as a bio-process in the wastewater treatment systems are scant and are applied on a minor scale. Algal systems are being practised in wastewater treatment either through the use of stabilisation

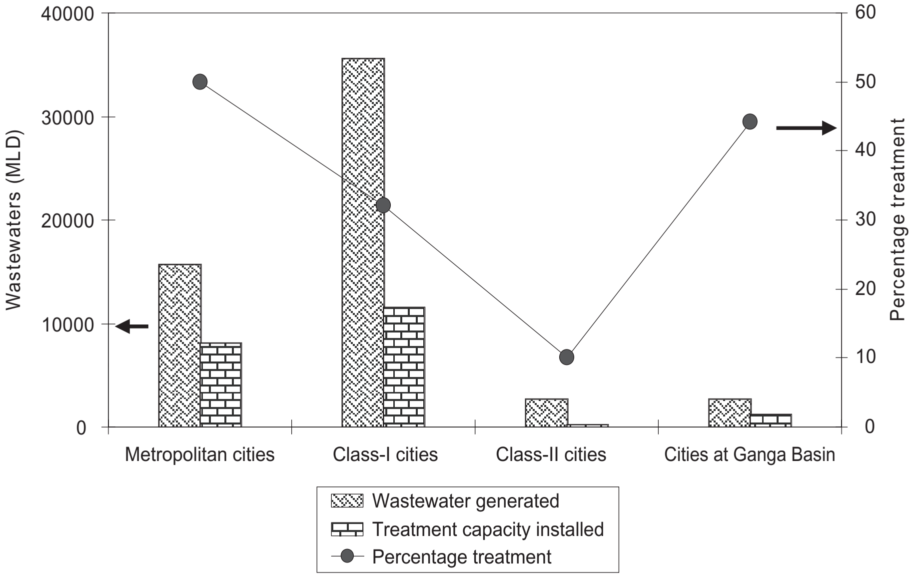

Figure 14: Wastewater generation in Indian cities.

(oxidation) pond based systems, and recently shallow well-mixed suspended algal raceway type oxidation ponds with mechanical mixing.[132]

A major requirement of wastewater treatment in rapidly urbanising developing countries is the removal of N and P, and to some extent C that forms the major cause for eutrophication of receiving water bodies. Most of the C released into the sewer system undergoes AD and is subsequently degraded aerobically during the period of its flow in open sewers and also storm water drains. Algae are efficient in removing N, P, organic toxicants and toxic metals from wastewater[14,148] and hence are key players for nutrient remediation particularly during the secondary and tertiary treatment phase of wastewater.

Significantly high rates of oxygen generation from photosynthetic algae (6–28 ppm) overcome the requirement of mechanical aeration systems which has a high initial and operational cost.[14] Oxygen in the dissolved form in treatment units enhances the rate of heterotrophic bacteria to remove organics.[149] Furthermore, algal treatment systems enhance the sustainability of the treatment systems by avoiding the generation of undesirable bi-products and secondary wastes such as sludge, thereby closing the loop for efficient nutrient recycling. Algal biomass in these ponds are rich in nutrients that can directly applied to land as low-cost fertilisers, as animal feed[141,149] or can be made to use in many different ways that satisfies our biofuel needs.

Much of the understanding of the algal dynamics and its wastewater ecology has been derived from laboratory based small pilot scale cultures and pilot scale high rate algal ponds (HRAP). There have been significantly higher numbers of studies on growth of algae under a wide variety of wastewater environments under different conditions. Most of these studies have focussed on urban wastewaters such as municipal sewage (Table 2) and dairy wastewaters, and have primarily examined the potential of algae to remove N and P, and in a few instances metals and other minerals from wastewater. Many studies have focussed on factors that are responsible for maximum algal biomass production and mechanisms of algal biomass harvest from wastewaters (Figure 15). This review attempts to provide insight to use wastewater algae for potential biofuel options.

3.9.3 Wastewater algal growth efficiency:

Algal growth and development in any set of cultivation systems depends on a multitude of variables, the physicochemical variables being pH, growth medium temperature, nutrient concentrations primarily C, N and P and other nutrients, light availability, gas exchange systems and efficiencies. Several studies have examined the factors affecting growth. And since algal growth studies in primary settled urban sewage significantly increased at longer photoperiods, addition of CO_2 and in the contrary increased temperature regimes during growth substantially decreased the density of algal biomass.[150]

Urban wastewaters are generally rich with high concentrations of C, N and P compared to the other growth media or water sources. The major fraction of N is in the form of ammoniacal–N

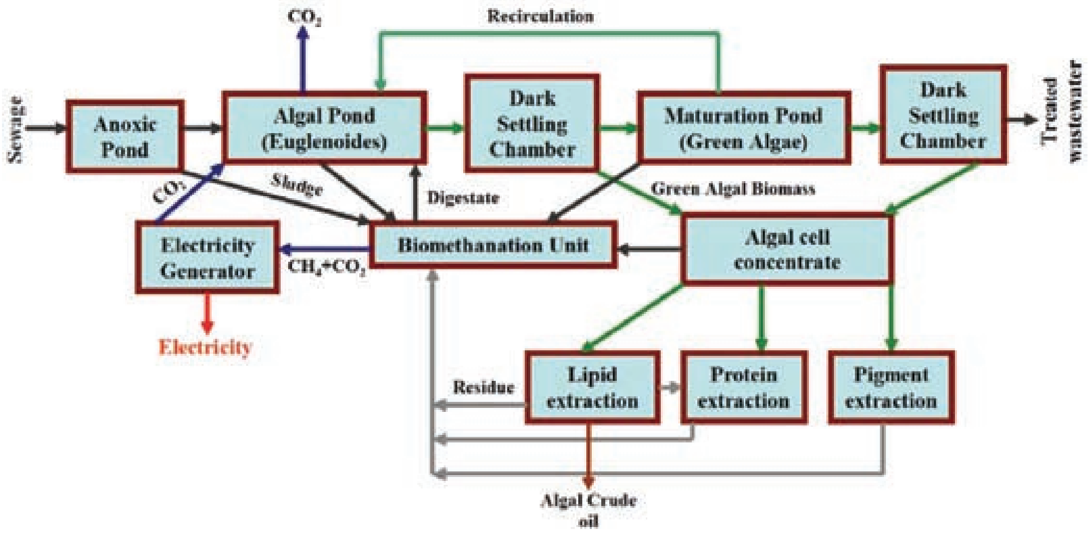

Figure 15: A flow-diagram showing how wastewater resources could be utilised for sustainable algal-based biofuel production coupled with wastewater treatment.

i.e. 35 ppm[138] which sustains many algal species dominated by Chlorophyceaen members comprising *Chlorella* sp., *Monoraphidium* sp. and *Chlorococcum* sp.[138] Contrary to this there are many studies that highlight the lethal effects of higher NH_4-N on algae due to ammonia toxicity.[150–152] The algal communities in an urban lake at Bangalore were reported to be responsible for about 76% N removal and 60% C removal.[137] Similar findings were also found in earlier studies where chlorophytic microalgae mostly comprising of *Chlorella* and *Senedesmus* have been shown to tolerate several wastewater conditions and were efficient at uptake of nutrients from wastewater.[15,153–160]

Chlorella and *Scenedesmus* are usually dominant among the phytoplanktonic communities in oxidation ponds[161] and in high-rate algal ponds.[162] The growth of *Chlorella* and *Scenedesmus* have been studied widely as these species grow well in sewage effluents.[157,163–166] These species show a high nutrient removal efficiency (>80%) and in many instances results in almost complete removal of ammonia, nitrate and total P from secondary treated wastewater,[159,167,168] indicating the potential of microalgae for tertiary sewage treatment. Among the chlorophycean members there can variations in the nature and rate of nutrient uptake in different species. *Chlorella vulgaris* was reported to be more effective than *Chlorella kessleri* in N and P uptake from wastewaters[169] while observations from other studies indicated that *Scenedesmus obliquus* grew better in municipal wastewater than *Chlorella vulgaris*.[159] Several other studies reveal the presence of euglenophytes in wastewaters with higher organic loading which derives nutrients from the environments mixotropically (CST, unpublished study).

3.9.4 Algal growth in municipal wastewater (sewage): In conventional treatment plants, N and P are usually removed in the tertiary phase of wastewater treatment systems where algae have been widely deployed and form the key agent for N and P removal. These algae could function either as free-swimming entities (in suspension) or in an immobilized form (Table 2). Studies conducted in different modes as under semi-continuos culture conditions reveal higher growth rates compared to batch cultures and this is attributed to the progressive nutrient depletion under batch conditions.[157] Other lab scale studies showed high growth rates and nutrient removal (90% N removal and 80% P removal) by deployment of algae on primary settled sewage with *Chlorella vulgaris* (without primary treatment).[163] Many of these batch operated lab scale experiments have shown high growth rates.[157] When the growth of the earlier study [163] is compared with that of *S. obliquus,* [157] the latter had higher growth rates and efficiency of nutrient removal which is attributed to the semi-continuous nature of the operation. However, *Chlorella* species were able to grow efficiently even at high nutrient loads (TN = 132 ppm; TP = 202 ppm) in centrate wastewater collected from from sludge centrifuge.[166] *Chlorella minutissima* isolated from wastewater treatment ponds in India had higher growth rates in raw sewage under pond systems compared to earlier studies.[165] This species was reported to grow heterotrophically in dark and had a mixotrophic mode of nutrition on variable C and N (ammonia/nitrate) sources at a wide range of pH and solids concentrations and has higher growth of 380 mg/l in heterotropic conditions compared to 73 mg/l under phototrophic conditions in 10 days.[165] These experiments indicate that chlorophycean members such as *Chlorella* as better nutrient assimilators in raw wastewater systems under laboratory conditions. However, under field conditions although chlorophyceans dominate the algal assemblages could vary a lot depending on the physico-chemical environment.[137]

Algal communities were found to be efficient in the removal of nutrients from agricultural wastewater and livestock waste slurries that are high in N and P.[140–142] *Botryococcus braunii* was reported to grow at higher growth rates in piggery wastewater comprising 800 ppm nitrates and and showed ~80% removal of nitrate-N.[142] Earlier observations reveal benthic algae as *Microspora willeana*, *Ulothrix* sp. and *Rhizoclonium hierglyphicum* possessed higher nutrient uptake rates compared to other algae in suspension.[170,171,14] Algal species have been also used to treat industrial wastewaters and have been thoroughly reviewed.[14,148] Algae such as *Botryococcus braunii* and *Chlorella saccharophila*, and marine alga *Pleurochrysis carterae* have been also found to grow abundantly in the carpet mill wastewater producing higher lipid yields.[135] Many algal growth and nutrient removal studies have been conducted on artificial wastewaters[153,174,154,157,158] that lack solids (organic matter) and do not adequately simulate the original wastewater conditions. These are simple systems with no nutrient competition and result in higher microalgal growth rates compared to performance in municipal wastewaters[157,163] which is attributed to higher toxicity and inhibitory effects of certain chemicals in real wastewaters or competition from resident bacteria, protozoans and zooplanktons.

Table 2: Studies on algal growth and biofuel production (lipid productivities) in various types of wastewater condition.

Wastewater	Algae	Cultivation period (day)	Biomass content (g/L)	Nutrients and COD (mg/L)	% Removal Nutrients/COD	Lipid cont. g/g.	Lipid prod. (mg/Ld)	Reactor type	References
Artificial wastewater	*Scenedesmus* sp.	15	0.25–0.33	NH_4–N (15.45–25.17); NO_3–N (14.4–16.86); T-N (29.86–42.03); PO_4–P (1.59–2.14)	73.4–79.4; 22.6–37.3; 48.9–65.5; 56.1–59.1	0.128	16.2	Batch	153
Centrate (sludge centrifuge)	*Chlorella* sp.	9	1.5	NH_4–N (71.8); T-N (131.5); T-P (201.5); COD (2250)	78.3; 82.8; 85.6; 83	– – – –	– – – –	Batch	166
Syntheteic wastewater	*Spirulina platensis* *Rhodotorula glutinis*	5	1.6	NH_4–N (165); COD (43,210)	35; 73	0.14	44	Batch	155
Treated industrial + sewage wastewater	Mixed species	10	4.9	NO_3–N (19.6–22.1); PO_4–P (19.6–22.1); PO_4–P (19.6–22.1)	– – –	0.07	–	Batch	135
Digested effluent	*Chlorella* sp.	21	1.7	NH_4–N (100); T-N (125); T-P (18); COD (820)	100; 78.3; 71.6; 34.3	0.14	11.3	Batch	166
Artificial media	*Scenedesmus* sp.	15		TN (2.5–25); T-P (0.1–2)	45–99; >99	0.3–0.5	190	Batch	156
Artificial wastewater	*Chlorella vulgaris*	2.5	0.38	TN TP	74.3; 70.2			Semi-continous	157
Artificial wastewater	*Scenedesmus obliquus*	2.5	0.4	TN TP	100; 60	0.16		Semi-continous	157
Urban wastewater	*Chlorella vulgaris*	9	0.19	TN TP	60; 80.3			Semi-continous	157
Artificial wastewater	*Chlorella vulgaris*	14	1.7	NH_4–N (20); T-P (4); COD (320)	99; 95; 59	0.33	40	Batch	158
Secondary treated wastewater	*Chlorella* sp. 227	9	0.67	T-N (19.1); T-P (1.2); COD (15.3)	92; 86; –24.8	0.31	22.9	Batch	159
Secondary treated wastewater	*Chlorella* sp.	9	0.41	T-N (19.9); T-P (1.3); COD (15.4)	85; 84; –13.8	0.15	6.9	Batch continous	159
Secondary treated wastewater	*Chlorella* sp.	9	0.5	T-N (20.0); T-P (1.3); COD (15.6)	75; 84; –13.9	0.22	13	Batch	159
Artificial wastewater	*Chlorella vulgaris* (FACHB 1068)	14	1.58–1.72	NH_4–N (20); PO_4–P (4); COD (400)	90; 94; 87	0.38	79	Batch	160

3.9.5 Potential lipid generation from wastewater grown algae: The capability of algal communities to thrive under acute conditions in typical wastewater illustrates that these nutrient rich wastewaters can be used as suitable natural growth medium for producing biofuel in a sustainable manner. These algal communities with higher growth rates and productivities can store significant levels of lipids in the cells. The nature and type of lipids (saturated, unsaturated, polyunsaturated and glycol/phospho lipids or TAGS) and the quantity of lipids produced (max-80% of the cell dry weight) depends on the nature of species and the algal growth conditions.[118–120] Reports from established sources reveal higher lipid concentrations from cells grown in photobiorectors or batch cell-culture in vitro. However, comparatively lower lipid concentrations are observed in open ponds and lagoons.[119]

The higher level of accumulation of lipids induced by stress induction (N and P limitation) are linked with low algal-biomass productivities.[7,123] The unlimited nutrient supply in nutrients wastewaters would result is high algal cell densities and higher lipid productivities. Several laboratory based studies focussed on biofuel from wastewater have been carried out in batch cultures, semi-continuous cultures and in bioreactors with ponds and have reported satisfactory lipid accumulation in wastewater grown algae yielding low (<15%), moderate (25–30%) lipid content and in many cases a very high lipid yield is achieved (Table 2).

Studies have reported an average lipid content of 12–18% (Table 2) depending upon the species selected and the growth environment. Algae mostly grown on municipal wastewater was found to have a biomass cells ranged from 20–35 mg/l/d with an annual biomas yield of 16–28 t/ha/yr to produce an estimated lipid yield of 3260–3830 L/ha/yr.[135] Batch culture growth studies[135] in municipal wastewaters and *Chlamydomonas reinhardtii* revealed higher growth rates with a lipid content of ~17%.[172] The same cultures being transferred to bio-coil showed a consistent growth in wastewaters with increased lipid content of to 26% and biomass productivity of 2 g/l/d and an estimated lipid yield of 0.505 g/l/d coupled with adequate nutrient removal.[172] Studies on *Botryococcus brauni* in secondary treated municipal wastewater showed higher lipid content (~18%) than conventional growth media (~11%). Such studies suggest a greater likelihood of lipid accumulation in wastewaters. Many more such types of investigations are required to evaluate the growth rates, lipid content and the lipid productivities in wastewater and synthetic media.[173] Contrary to the above another study has reported higher growth rates (thrice) along with significant decrease in total fatty acid content (9 mg/g) compared to control cell cultures (46 mg/g) during the same cultivation period.[174]

Many other studies have also shown the potential of algal biofuel production in different kind of wastewater, livestock (dairy) manure wastewater, etc. The lipid content from mixed algal cultures, isolated from sewage treatment ponds, grown in anaerobically digested dairy manure wastewater in outdoor batch cultures showed a leap in lipid accumulation in a week (14% to 29%) with an estimated lipid productivity of 2.8 g/m^2/d.[135] *Chlorella* sp. grown in batch culture on anaerobically digested dairy wastewater showed total fatty acids ranging from 9% to 13.7% with varied wastewater concentrations and producing a cell dry weight picking up from 1.47 to 1.71 g/l giving a lipid productivity of 11 mg/l/d.[166] *Chlorella* sp. grown in dairy manure wastewater were compared with two setups with suspended growth and polystyrene foam substratum growth medium which showed equal lipid contents (9%) in both the systems, however, algal biomass yield were higher in attached algae with a fatty acid yield of ~2.6 g/m^2 accounting to a fatty acid productivity of 0.23 g/m^2/d.[175] The study on *Rhizoclonium hieroglyphicum* grown turf type pond system compared different types of wastewaters with or without CO_2 supplementation and revealed higher lipid content in pig-wastewaters grown algae (9.3%), however the algal biomass productivity was greater in dairy effluent (21.3 g/m^2/d dry wt. basis) compared to other wastewaters (10.7 g/m^2/d dry wt. basis) of the pond scale conditions with certain inconsistencies, that lead to higher lipid productivities 156 mg/m^2/d (without CO_2 supplementation) and 210 mg/m^2/d (with CO_2 supplementation) with just 86 mg/m^2/d from pig wastewaters.[170]

3.9.6 Challenges and limitations for potential algal wastewater biofuel production

Efficient biomass to biofuel conversion technologies: There are several processes and mechanisms of biomass conversion to biofuel for energy generation. What becomes important is use of the technically viable and efficient methods for maximum biofuel yield. The algal biomass-to-energy conversion processes vary with the nature, type and sources of algal biomass and end-use.[176] These technologies can be broadly divided into two basic conversion systems thermochemical and biochemical conversion. A multitude of factors affect the options for selection of conversion technologies, mainly the type and quantity of biomass feedstock; desired form of energy; cost-effectiveness and net economy; end-form of the product.[177] The thermochemical technologies involve the thermal decomposition of organic components in biomass to yield fuel products, and are attainable by various processes such as thermochemical liquefaction, pyrolysis, direct combustion and gasification.[178] However the biochemical processes of conversion of the crude form to fuels can be categorised into two: anaerobic digestion and alcohol fermentation.[22] Although sustainability of the system depends, to a large extent, on the algae to biofuel conversion technology and its efficiency as well as appropriateness for the location and end-use, the subject is quite vast and deserves a separate review, and is therefore not discussed in detail. It is however important to recognize that these technologies need to function at both decentralized scales of villages as well as at a higher centralized system as planned for large tracts of saline land cultivation.

Efficient algal harvesting techniques: There has not been much progress in the downstream processing of algae especially in the evolution of adequate cost effective methods in harvesting; concentrating, (partial) drying and temporary storage of algae from algae based wastewater cultivation units. To enable sustainable biofuel production it is important to have many options for decentralized algae harvesting in situations such as the wastewater treatment plant, paddy field algal cultivation or coastal shelf cultivation of macroalgae. The harvesting becomes challenging especially when algal cells are small and densities are low in open pond type systems. This could be one of the major reasons for less extensive use of algae based wastewater treatment and is disadvantageous from pond biomass recovery point of view, which nevertheless needs to be addressed.[144]

The first stage in the harvest of algal mass from open ponds is enabling flocculation of the cells to increase their peak growth, measured as cell densities, reaching 10^4 to 10^7 cells/ml to an algal cell concentrate having about 20–100 g dry mass/litre of algal mass or slurry. Later on this mass could be concentrated further, e.g. by centrifugation, to achieve a higher mass that could facilitate easy drying for lipid extraction. Centrifugation, filtration and gravity based settling are presently used for harvesting algal biomass after the flocculation step.[179] Potash alum has been extensively used in the final polishing ponds.[179] On the other hand, algal species naturally aggregate at a certain stage of maturity and often spontaneously and settle to the pond bottom which can then be harvested easily.[32] Among many other systems these clumps come up to the surface aiding mechanical algal harvest. This natural auto-flocculation or bio-flocculation mechanisms needs to be utilized to enable low energy algal harvest from wastewater treatment plants. It is reported that algal bio-flocculation is triggered in a few species by C limitation or oxygen deprivation and various other environmental stimuli.[165] These need to be adopted in the wastewater algae production and biofuel production technology to reduce the energy sacrificed for algae recovery. Consequent to harvesting the algal mass is usually concentrated either by centrifugal[39] or gravity sedimentation methods that are current practises.[179] Filtartion processes may also be employed for algal cell harvest and concentration. Vaccum pressure filtration aided by use of various porous media are suitable for the recovery of larger algae (>70 μm) but the process becomes inefficient for *Scenedesmus* and *Chlorella* (5–20 μm), i.e. smaller microalgae.[54] This segment of algae production needs to be evolved for various types of algal cultivation discussed earlier in this article.

3.9.7 Sustainability of biofuel generation from wastewaters through algae: Potential for Bangalore: A positive net energy gain is a critical factor for algal biofuel production even from treating and managing wastewater of a city. This offsets a part of the costs of urban wastewater treatment. These components could make this approach an effective algal biofuel option while sustainability would be enhanced by a three-fold approach to sustainability. Firstly, algae based wastewater treatment without simultaneous production or emissions of methane would reduece the GHG and C-footprint. The commonly practiced wastewater treatment involves the use of a lot of fossil fuel based power—coal power which will be avoided and reduces the environmental footprint of the system. Thirdly, processing of algae would provide a green fuel that could possibly capture a part of the CO_2 emitted elsewhere. In this way there is potential for triple benefits that could be appropriately packaged for the C-markets. A number of studies conducted on life cycle energy assessments of different biofuel substrates and feed-stocks report positive energy gain in microalgal systems with poorer performance compared to other biofuels. Studies reveal crop-based biofuel having lower energy use, emissions and water use compared to algal biofuels.[180] There is certainly a big gap between the modelled and actual environmental performance in achieving the energy targets by algal biofuels mostly due to land, water and nutrient requirements added to costs associated with the downstream processing. Yet, wastewater processing using algae and algal biofuels is an interesting challenge in this area.

The city of Bangalore produces nearly a 1200 million liters per day (MLD) of sewage that could be processed as indicated above to achieve the triple benefits of algal biofuel production, GHG emission reductions (and CDM attached to it) as well water purification for reuse. It has been reported earlier that due to the mismatch between the rate of growth of the city and the rate planned by the city planners, there is a short fall in the capacity of the wastewater treatment plants set up in and around the city. This wastewater therefore, after flowing along the stormwater courses, enters many of the man-made water bodies previously used for crop irrigation. It has also been reported earlier that these water bodies now function as wastetwater treatment plants with low C-footprints. The urban lakes (manmade waterbodies) in Bangalore now become rich in nutrients fed through domestic sewage and resolve into three zones—anaerobic, anoxic and algae-dominated oxic zone, such that when water exits these waterbodies, the wastewater, sewage, entering these lakes are treated to near standards needed by the environmental enforcement agencies. Algae are the dominant species that make this functional.[137] A similar set of findings are also observed in earlier studies.[149] The wastewater systems provide a year round availability of nutrients with a rich natural microflora for a synergistic growth of algal-bacterial systems with adequate nutrient load.[103,137] The manmade lagoon system removes the burden of land area requirement for wastewater treatment while it also provides space and options to grow algae for biofuel and wastewater treatment.

Considering the case of a model city like Bangalore, nearly 1200 MLD of wastewater in generated daily that flows through the underground sewers and storm water drains and finally reach the cascading system of water bodies in the city by virtue of slope, gravity and drainage basins. Studies carried out earlier shows that one of the catchment, the Bellandur-varthur stream draws ~500 MLD of sewage into these two large waterbodies. They also reveal adequate treatment of wastewater possible by natural micro and macroflora (algal-bacterial systems) with an efficiency of 50% nutrient removal and 70–90% C-removal. The average algal cell densities are of the order of 10^5–10^6 cells/ml with biomass productivities of 8–12 g/m^2/d. Such systems can be optimised and made sustainable by careful feed rate management and algae recycling. Investigations on the nutrient loads showed higher influx of nutrients i.e. 90–120 mg/l TOC; 75–90 mg/l TN and 20–40 mg/l of TP, entering the first lake system. The average annual influx of nutrients amounts to 20,000t TOC/yr of, 14,600 tTN/yr of and 5,475 tTP/yr. Such high nutrient levels in these systems has triggered extensive algal proliferation with many a dominnat algal genera. The biomass generation potential for 380 ha water body would be ~13,000t algal biomass/annum. Out of this if only 40% in harvestable algae is recovered, it then yields ~5000t algal biomass/yr. Considering an average lipid content of 20%, a 1000 t of lipid can be extracted from this water body per annum. This benefit is over and above methane emission avoidance as well as fossil fuel energy expenditure.[103]

The total generation of wastewater in Bangalore is ~1200 MLD. For the entire wastewater the net lipid potential corresponds to 7,200 t/yr. During the lipid extraction step a huge quantum of disrupted cells and cellular constitutuents are left behind. This spent algal-biomass generated can be anaerobically digested that would add to additional energy security from the spent biomass by AD. Considering that 50% of the algal harvest (~18000 tonnes/yr) being anaerobically digested, would generated 7 Mm^3 of methane at a modest biomethanation rate with an energy equivalent of 2.8 GWh. The slurry left after the anaerobic digestion can be used as an excellent nutrient media for algal cultivation or can be used as rich to fertilisers which would take a step further to stabilise our agrarian economy. In this complete loop there is no net generation of waste at the same time there is a positive net energy gain that indicates sustainability (Figure 16). Further investigations on such type of naturally managed system and its process understanding will eventually lead to optimal wastewater treatment with efficient biofuel production. From the earlier notes it is evident that the algal biomass can be transformed to biofuels via a wide range of technologies. However, lipid extraction from algal biomass as an oil source for biodiesel is the most promising option, particularly when cogeneration of methane from the remaining residual algal biomass becomes possibleand viable.[181]

3.9.8 High algal biomass productivities from urban wastewater fed lakes and water bodies (pond scale): Albeit the lower average lipid content of algae in wastewaters, the enormous and consistent nutrient supply results in high algal biomass productivity leading to increased lipid yields. Many studies in wastewater have shown higher biomass yield[172] mostly in lab and pilot scales studies that do now provide adequate assurance

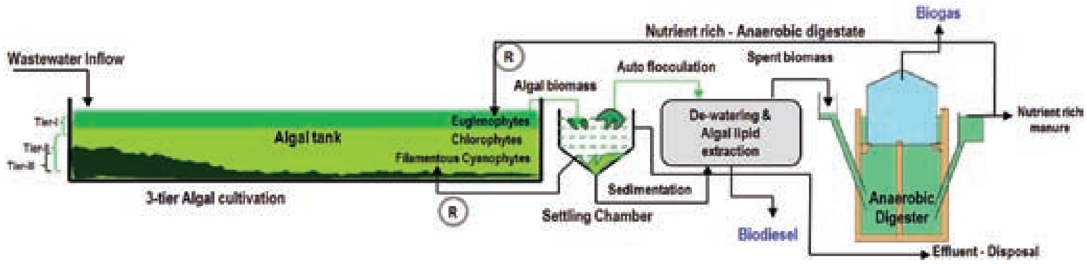

Figure 16: Schematic diagram of a wastewater treatment with biofuel production for Indian cities.

for yields at commercial scale of operation. Initial studies of wastewater-grown microalgae have reported very significant biomass yields[15,155,160] and promises as well as suggests that future work must demonstrate similar yields on a larger scale and in open pond conditions. Although exhaustive studies have been carried out on algae and their growth rate, most of these studies have been performed under controlled laboratory conditions and often in batch mode (in different types of shake flask or bioreactor systems). However, there are very few studies of growth and lipid yields under field conditions for large open pond type systems for algal mass cultures. The studies performed earlier need to address long term viability of the algal species under varying weather and environmental conditions and ensuer stability of the algal populations with consistent biofuel yields. There are also many reports that attempt genetic manipulation of algae[15] for lipid accumulation, however, no stable transformation have been achieved for a sustainable biofuel production till date—these are often not permitted for this purpose and other options need to be examined. Many other studies have proposed alternatives of improving the biomass generation and lipid yield by a process called photosynthesis and fermentation process for algal cultivation[10] where the studies evaluated the integrated strategy of algal cultivation with an initial autotrophic growth phase where high biomass is generated, followed by a heterotrophic fermentation phase to maximise cell density and lipid accumulation. The results showed increased lipid yield (70%) of *Chlorella protothecoides* representing a higher conversion efficiency of sugar to oil during photo-fermentative growth. Earlier investigations show higher cell densities at phototrophic conditions with lower lipid accumulation; however heterotrophic algae like *Chlorella protothecoides* have higher biomass productivities as well as higher lipid content/accumulation.[182] These studies would especially lead to improvement in the lipid productivities of algae that are grown in highly organic carbon rich wastewaters.

With the current technological advancements and methods of lipid extraction, algal biofuel generation might not yet be economically feasible with inadequate postive energy returns. Coupling wastewater treatment with biofuel production can be an attractive option for deriving bio-energy at low cost, with reducing GHG emissions, nutrients (fertilisers for algal growth) and cost of freshwater resources for growing algae yielding biofuel. Sustainable renewable energy can be drawn consistently from the wastewater grown algae as higher biomass productivities enhances the feasibility of consistent biofuel generation. Algal biomass harvest can be optimised to get higher yields by altering the physico-chemical environments as CO_2 addition, species control at every zone, control of grazers and pathogens and standardizing the natural bio-flocculation ability of algae to suit to tropical systems that improves the economic suitability for the mass culture. The total biofuel potential of algae, when grown in domestic wastewaters generated by 1000 urban-centres in India is ~0.16 Mt/annum considering the lipid fraction as 20%. This renewable can supplement to the present fuel needs for transports and can reduce imports fostering sustainability for the nation.

3.10 *Threats to large scale cultivation*

As of now, large scale microalgal cultivation appears to be an environmentally sustainable option to produce alternative biofuels. Large scale microalgal cultivation does not compete with food crops for fertile land as such facilities could be established on dry and waste lands and coastal areas and can utilize saline/brackish and wastewaters. Microalgal cultivation also has the potential to sequester CO_2. However, at present the large scale cultivation of microalgae for biofuel purpose are not economically competitive and require detailed R&D efforts. The major threat to microalgal approach to biofuel could be from other competing technologies which may be developed in future for conversion of renewable resources and waste materials to biofuel.

3.11 *Future work*

A lot of laboratory and pilot studies have shown the potential and feasibility of algal biofuels under controlled conditions. The obvious future path would be to adapt and establish cultivation and algal fuel recovery under typical field conditions, especially under conditions of flooded paddy, large sea water cultivation on saline wastelands and mass cultivation in fishery deficient coastline. This next step is also expected to raise several sustainability questions and challenges for which appropriate solutions would emerge. Some of key areas where research thrust would be of immense developmental value are

1. Exercise of strain improvement; strain development and evolving competitive species mixes will lead to having a good stock of potential algae adapted to Indian situations and possessing high potential for lipid accumulation (including lipid enhancement options) under typical Indian conditions.
2. Field testing of the cultures and species mix under a variety of field conditions and resultant adaptation.
3. Simplification and decentralisation of the algal cultivation technologies starting from inoculum maintenance, technologies to provide startup cultures in villages; standardization of open pond and paddy plot cultivation technologies.
4. Use of low energy and appropriate technologies for decentralised
    i. Algal harvest with liquid recycling,
    ii. Concentrate and process algal biomass for lipid and biomethane and
    iii. Recycle nutrients with low losses.
5. Long range testing and evolving standards using algal biofuels (in engines) and byproducts.
6. Control of pest and predators of various algae under different field situations.

4 Conclusions

Algal biofuels has been considered as a sustainable and environmentally clean fuel for the future that is C-neutral. Without an effective policy and control, large scale algal biofuel production is likely to impact food security and therefore needs to be cultivated in areas that do not conflict with food security. Four types of tracts that can support reasonably high algal productivity without threatening food security have been short listed as

a. Multi-tier cropping within flooded paddy
b. Hyper saline algae cultivation in inundated salt flats of western India
c. Urban domestic wastewater and
d. Fishery deficient coastal areas.

Much of the laboratory scale promise of high algal lipid yields now needs to be converted to feasible and viable technologies suited to the above four tracts and field tested, only then various finer issues of sustainability can be closely monitored. However, in general it is clear that algal biofuels will somewhat increase demand for water and require novel techniques for multiple recycling of plant nutrients without losses so as to ensure that using the same pool of available water and plant nutrients both conventional crop as well as algal biofuels can be raised and remain sustainable. A lot more research and technology develop is required in areas of algal cultivation, harvest and processing before large-scale substitution of PDF by algal biofuels can be realized. Waste management efforts can be efficiently adapted to algiculture and algal biofuel production to fufill the dual opbjectives of envinonmentally benign wastewater processing and C neutral biofuel production. This requires that multiple goals of biofuel production, sustainability and waste management be synchronized.

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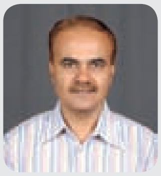
H.N. Chanakya has a PhD in Microbiology from UAS Bangalore and has been working in the field of anaerobic digestion of wastewater and wastes as well as drinking water purification for over 30 years at the Centre for Sustainable Technologies, Indian Institute of Science, Bangalore. He has developed over eight technologies in the field of sustainable energy and water. In the area of wastewater his research interests lie in evolving low energy and sustainable wastewater/grey water treatment coupled to energy and nutrient recovery including the use of mixotrophic algae. He also helps the Karnataka State in an advisory capacity for its mandatory and regulatory bodies in the area of wastes and wastewater management.

Durga Madhab Mahapatra is a research scholar at Centre for Sustainable Technologies, Indian Institute of Science (IISc), Bangalore. He received his Bachelors in Technology degree in Biotechnology (2003-2007) from MITS, Rayagada, BPUT, Orissa. His research interests include bioprocesses engineering, wastewater microbiology and ecology, environmental biotechnology, bioremediation and applied phycology.

R. Sarada obtained doctoral degree in Biochemistry from Mysore University in 1992. She is working at CFTRI as Scientist from 1991 and currently holds Senior Principal Scientist position. She has 20 years of R&D Experience in the area of Algal Biotechnology and Food Biotechnology. She has handled several R&D projects and has carried out extensive work on commercially important algae such as *Haematococcus, Botryococcus, Dunaliella, Porphyridium*, and other indigenous microalgae for lipids and bioactive molecules. She has published 55 research papers, 14 patents and few book chapters. She received best scientist award at the Institute and obtained best poster and paper awards in different symposia and conferences, she is a member of National Academy of Sciences, India. She is also involved in transfer of technology to entrepreneurs.



V.S. Chauhan obtained doctoral degree in Bioscience from R.D. University, Jabalpur, MP in 1991. He is working at CFTRI as Scientist from 1999 and currently holds Principal Scientist position. He has 15 years of Experience in the area of Algal Biotechnology and Business Development. He was involved in setting up of one India's largest *Spirulina* production plant at Nanjangud in private sector. He is involved in R&D projects on microalgae at CFTRI and also in the commercialization of knowledgebase of the Institute. He is involved in the dissemination of CFTRI technical services to various industries and entrepreneurs.

Ms. Abitha R is a Research Scholar at the Centre of Sustainable Technologies, IISc, Bangalore. She received her Bachelor's in Engineering in Environmental Engineering and after a short research assignment of working on algae at CFTRI Mysore, she joined CST, IISc. Her research area and interests are in raising algal biofuels, especially with fresh water algae under flooded paddy conditions.