Pre- and Post-digestion of microalgae makes an efficient energy product (Biogas)

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Imagine the world without petroleum products; it seems like a nightmare where the lives will be stuck in the darkness of depleting natural energy resources and its geographical availability. We are dependent on petroleum (oil-based and gaseous) for jet fuel, light vehicle fuel, heavy duty vehicle fuel, machine engines and for cooking purposes. These applications cover large sector of industries and households making it responsible for country's economy in a huge way.

A new era of **biofuels** derived from plants and microalgae has now achieved its milestones when the biofuel powered aircrafts have undergone successful flights in India and abroad. In terms of gaseous fuels, biomass-derived bio-methane or biogas has also been tested as the only fuel source to drive a vehicle in IIT Delhi, India.

Among biological substrates for fuel, microalgae possess a great potential for biofuel generation as it has high oil content and high carbon to nitrogen ratio. It also has potential to grow and treat waste waters and also fixes atmospheric CO_2. Hence, looking at these diverse applications, microalgae can be considered to be one of the most purposeful micro-organism for industrial applications.

Apart from being so useful for industrial purposes, the major limitation in using microalgae for biofuel route is separation of biomass from liquid media (dewatering) and poor digestibility of algal cell wall. The potential dewatering techniques have been critically compared by author in the form of a chapter in book entitled '**Algal Biofuel**' published by Springer International in 2016.

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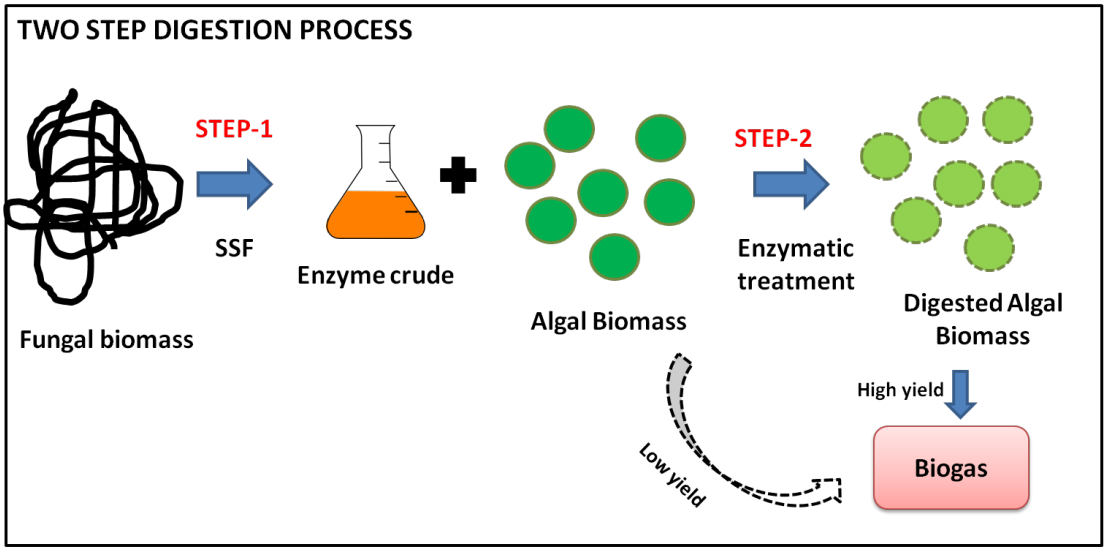
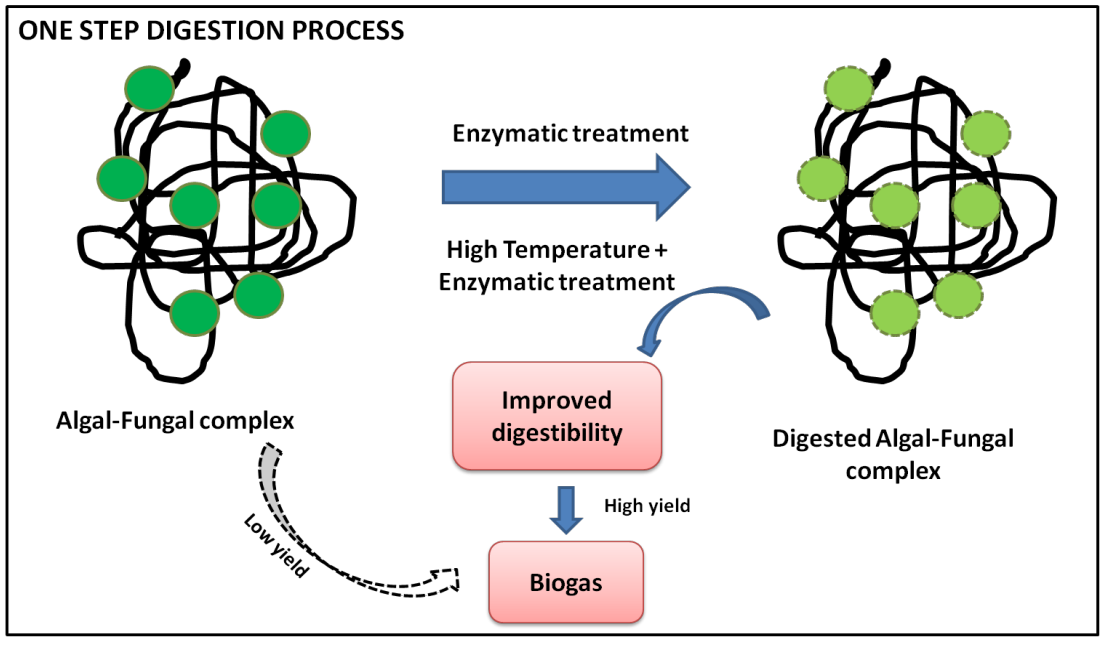
To solve the above two problems, the authors have developed a novel process, which efficiently solves the discussed issues of microalgal biofuel route. In the study, the algal biomass is harvested (dewatered) as well as digested within the same system or environment by making use of another biological organism i.e. fungus.

In our study, dewatering issue of microalgae was addressed using a fungus from *Aspergillus* family i.e. *Aspergillus fumigatus,* which was grown in the form of mycelial pellets (tiny thread-like balls of fungus). Allmicroalgal cells were allowed to attach on fungal thread-like mycelium within 4 hours under certain condition, which resulted in the formation of an algal-fungal complex (AF complex). This algal-fungal complex was big and heavier enough to settle down quickly without any application of any external force. This process was then optimised for best conditions relating to maximum/complete algal biomass recovery in the form of algal-fungal complex and was published in ***Algal Research*** journal in 2017.

The digestibility problem of the microalgae was solved by fungus exposure in two different ways. At first, a two-step process was employed, where the fungus *A. fumigatus* was used to produce enzyme crude using sugar bagasse as cellulose substrate under solid state fermentation. The enzyme crude showed a very high cellulase activity (103 FPU/g), which was then subjected to algal biomass to be acted upon by fungal enzyme. The enzyme activity was so high that even five times diluted enzyme crude was able to kill almost 100% of the algal cells when exposed till 24 hours of incubation at a temperature of 38°C. The dead cells and the live cells showing different fluorescence were distinctly counted using contrast colours by an automated fluorescent cell counter. The release of sugar, as the breakdown product of cellulose, was also found to be 92%, inferring a high level cell wall digestion. Although, this process was highly efficient to digest the microalgal biomass, but to make this a single-step process, another approach was adopted.





According to this approach, the algal-fungal complex formed were directly subjected to simultaneous enzyme production and pretreatment of microalgae for its digestion. Since both of these biomass were in a very close proximity, it was quite easy to provide such a condition, which is favourable for fungus to secrete cellulase like enzymes using microalgal cell wall as its cellulose substrate. On the contrary, it became unfavourable to microalgae as the action of fungal enzymes lead to algal cell wall breakage, which is mainly made up of cellulose. According to the viability assay, both the organisms (algae as well as fungus) were completely viable after formation of algal-fungal complex. However, the purpose was to kill or digest microalgal cell by the action of cellulase-like enzymes secreted by live fungal biomass attached to it. Hence, to complete this activity, the algal-fungal complex was incubated at two different temperatures, i.e. 38°C (optimum for cellulase production) and 55°C (optimum for cellulase activity) for 3 days. Such high temperature i.e. 55°C was chosen for two reasons: (i) to provide an additional heat pretreatment to AF complex for better digestibility; (ii) to provide optimum temperature for real time and efficient cellulase activity. As control sets, algae and fungus were also incubated individually under similar condition.

According to the visual observations, the AF complex and algae control at 55°C showed brown colouration of algal biomass instead of green color within 24 hours of incubation. This indicates the onset of algal digestion due to high temperature and cellulase activity. The enzyme activity, at different time interval using Whatmann filter paper as cellulose substrate, was also highest in 55°C incubated AF complex followed by algae alone (55°C), AF complex (38°C) and algae alone (38°C) after 3rd day of incubation. As cellulose is made up of multiple monomer units of glucose, therefore, the digestibility of algal cell wall was also observed in terms of sugar released

after cellulose breakdown. The sugar release in all the experimental sets followed the same trend according to the quantity of enzyme produced after 3 days i.e. more enzyme, more was the sugar release. When these digested AF complex (biomass) were tested for biogas production for 30 days by anaerobic digestion (with co-digestion of cow dung), the AF complex showing highest degree of digestibility (at 55°C) was able to produce 309 ml per g VS_{fed} cumulative biomethane. This amount of biogas produces contributed to 23%, 30% and 35% increased biogas in comparison to AF complex at 38°C, algae alone at 55°C and algae alone at 38°C, respectively. This quantity of biogas produced is much higher in comparison to the biogas produced by the conventional substrate of biogas i.e. cow dung. Hence, the co-digestion of pre-digested algae with cow dung appears to be the most potent biomass for biomethane production as fuel product.

To summarize, the study provides a new ray of hope to the biofuel industries for using microalgae as a new feedstock.The develop process unravel the hurdles related to microalgae, which was hampering its use as a commercial biofuel substrate. The advancements and new modification in the conventional methods for biogas production may lead to a revolution in the energy industry. Hence, further scale-up and its optimisation will bring us near to the implementation of this technology for fuel generation applicable to number of vehicles and for cooking/burning purposes.