**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 deal with a large sector of industries and households, which makes it a huge share responsible for country's economy.

A new era of **biofuels** derived from plants and microalgae 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.

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 wastewaters along with fixing atmospheric $CO_2$. Hence, looking to these diverse applications, microalgae can be considered to be one of the most purposeful micro-organism for industrial applications.

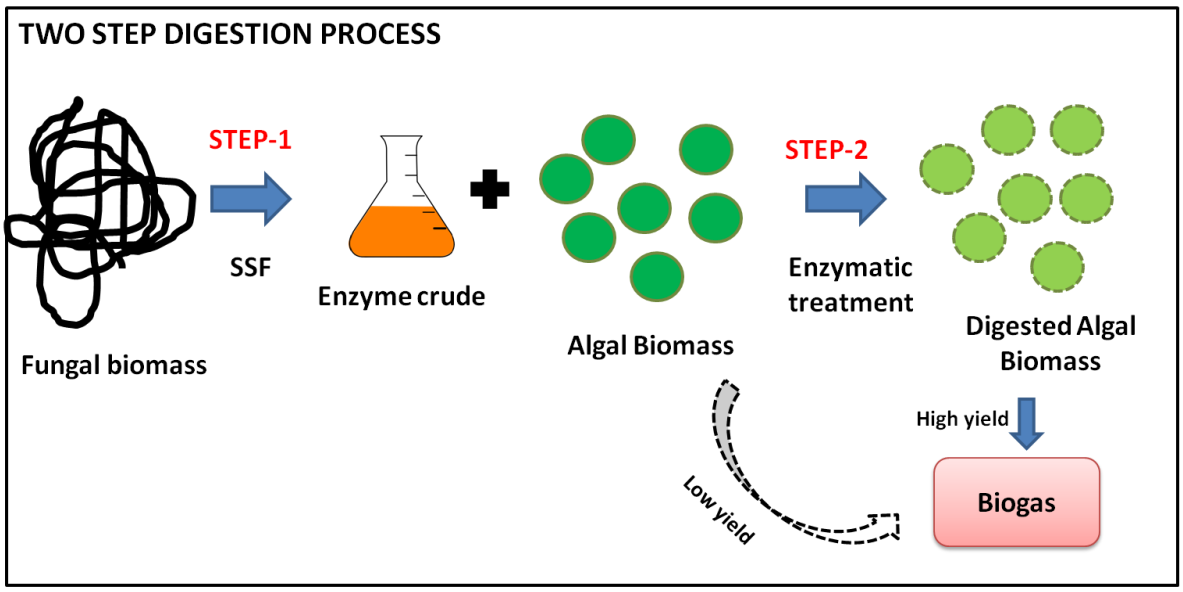
Apart from being so useful for industrial purposes, the major limitation in using microalgae for biofuel route is separation of biomass from liquid media (dewatering) and poor digestibility of algal cell wall. The potential dewatering techniques have been critically compared by author in the form of a chapter in book entitled '**Algal Biofuel**' published by Springer International in 2016. To solve the 2 above said problems, the authors have developed a novel process, which solves these two issues of microalgal biofuel route very efficiently. In the study, the algal biomass get harvested (dewatered) as well as digested within the same system or environment by making use of another biological organism i.e. fungus.

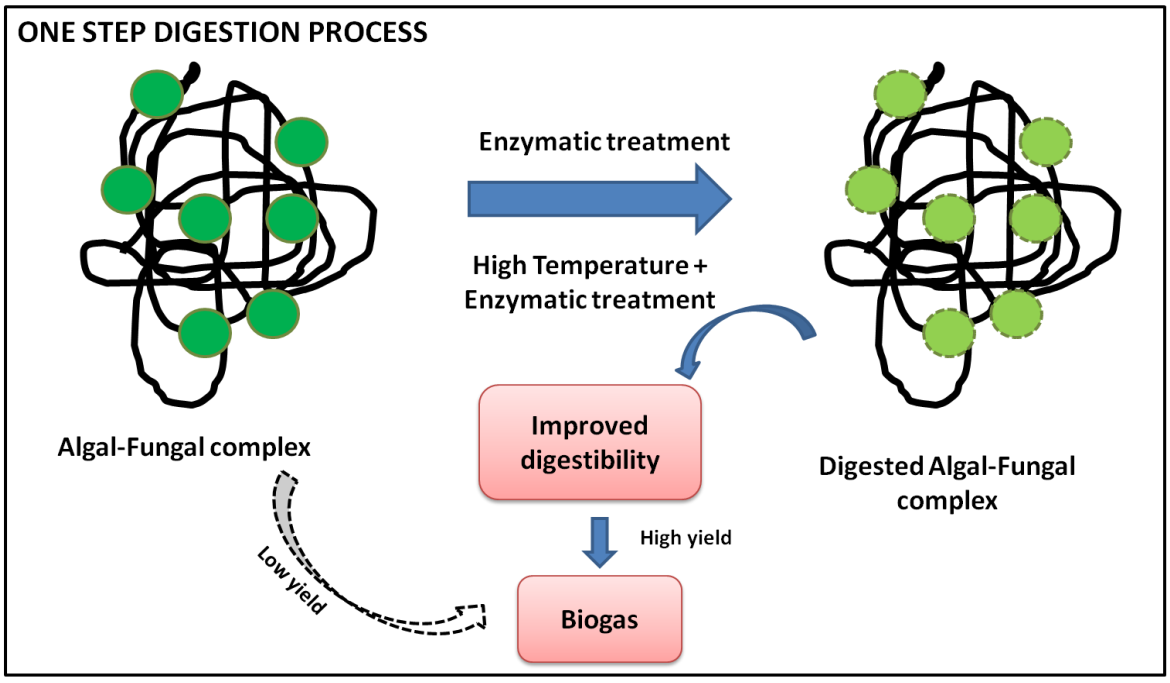
In our study, a fungus from *Aspergillus* family i.e. *Aspergillus fumigatus* were grown in the form of mycelial pellets (tiny thread like balls of fungus). All microalgal cells were allowed to attach on fungal thread like mycelium within 4 hours under certain condition, which resulted in the formation 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 optimized 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 2-step process was employed, where the fungus *A.fumigatus* was separately 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 action. The enzyme activity was high enough such that even 5 times diluted enzyme crude was able to kill almost 100% of the algal biomass when exposed till 24 hours of incubation at a temperature of 38°C. The dead cells and the live cells were distinctly counted using contrast colors by an automated cell counter. The release of sugar as the breakdown product of cellulose was also 92%, inferring a high level cell wall digestion. Although, this process was highly efficient to digest the microalgal biomass, but to make this process from 2 steps to 1 step, another approach was adopted for microalgal digestibility.

According to this approach, the harvested algal-fungal complex formed after harvesting algae by fungal mycelia pellets were directly used for simultaneous enzyme production and pretreatment of microalgae for its digestion. Since, both of these live biomass were in the close proximity, it was quite easy to provide such a condition, which is favorable for fungus to secrete cellulase like enzymes using microalgal cell wall as its cellulose substrate. On the contrary, it became unfavorable 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 digestive or cellulase like enzymes secreted by live fungal biomass attached to it. Hence, to complete this activity, the algal-fungal complex was incubated at 2 different temperatures i.e. 38°C (optimum for cellulase production) and 55°C (optimum for cellulase activity) for 3 days. The temperature as high as 55°C was chosen for 2 reasons: (i) to provide an additional heat pretreatment to AF complex for better digestibility; (ii) to provide optimum temperature for realtime and efficient cellulase activity. As control sets, only algae and only fungus were also incubated under similar condition.




According to the visual observations, the AF complex and only algae control at 55°C showed brown coloration of algal biomass instead of green color within 24 hours of incubation, indicating 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 3$^{rd}$ 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 as that of 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, contributing to 23%, 30% and 35% elevated 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 cowdung appears to be the most potent biomass for biomethane production for fuel application.

To summarize, the study provides a new ray of hope to the biofuel industries for microalgae as a new feedstock as the develop process looks after the hurdles in using this organism as a commercial 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 optimization will bring us near to the implementation of this technology for fuel generation applicable to number of vehicles and for cooking/burning purposes.