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Nutrient recovery and energy efficient algal harvest from anaerobic digestor wastewater

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ABSTRACT: The increasing levels of generation of wastewaters which are nutrient rich pose serious issues where conventional biological and chemical methods of treatment have failed in meeting sustainability challenges. In this study naturally occurring mixed algal species reared in mixotrophic growth modes have been deployed to remove recalcitrant organics and recover high nutrient concentrations (N and P) from anaerobic digestor wastewater at short residence times of 6-7 days. Results from pilot scale operation show that the cultivation methods adopted and the use of naturally selected species lead to a tendency among these species to clump at certain stages of growth that in turn float or settle rapidly making algal harvest and thereby the nutrient recovery processes energy efficient. Algal biomass productivity in the liquid from anaerobic digestor of the consortia varied with season with a maximum of 6.3 g/m^2/d.

Keywords: Mixed algal culture, Nutrient recovery, Harvesting

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1. Introduction:

1.1 **Nutrient recovery**: Today nutrient removal and recovery are important aspects for sustainable wastewater processing. The extent of which becomes alarming when we look at the fertilizer production and import figures for India. In the year 2010-2011, 66 lakh tons of urea and 131.8 lakh tons of other fertilizers were imported amounting to INR 1,50,000 crore expenditure. Hence even if half the nutrients are recovered, it would ease the situation tremendously. Conventionally wastewater treatment systems attempt to absorb and respire decomposable organic carbon removing the 'polluting' plant nutrients N and P by mineralization process, volatilization and recover a part of these as cell biomass, typically in bacterial sludge. Algae on the other hand offer many advantages to be deployed as a treatment option for wastewaters rich in nitrogen and phosphorous. They provide dissolved oxygen (via photosynthesis) to the accompanying bacterial population without input of external energy [1, 2]. Algae take up ammonia and nitrate [3], facilitate phosphorous removal and recovery by uptake, adsorption and precipitation [4, 5]. They also contribute to removal of organic carbon by mixotrophic mode of nutrition, in the process often ridding the wastewater of toxic and/or recalcitrant organics [6].

Algae mediated wastewater treatment system have been used as oxidation ponds, anaerobic lagoons etc. However, the processes that are currently deployed involve algae mediated treatment and mainly consider the treatment of such wastewaters without options to harvest the algal biomass grown. This is often due to the absence of technology options for energy efficient harvesting, dewatering and drying of the algae raised by the system. This property becomes a physical limitation and makes the process of wastewater treatment most often uneconomical and also unsustainable due to a variety of reasons.

In this research work an attempt has been made to address this issue and develop strategies for the simultaneous wastewater treatment and energy efficient harvesting of algae grown to enable further applications. Such a process is expected to recover the key plant nutrients of N and P from the wastewater making the process sustainable

1.1 Importance of nutrient recovery:

Human activities that increase the nitrogen being fixed include production of nitrogen fertilizers and cultivation of nitrogen-fixing legumes (together account 80% of nitrogen added) and burning of fossil fuels (20%). The nitrogen being added is not spread evenly across the earth but there is no place on Earth that is unaffected. The effect is more so on the Indian subcontinent and south east Asia where crop production is more intense. Though nitrogen is a nontoxic gas, its intermediates (as reactive nitrogen species) present in the atmosphere influences the oxidation capacity, the radiation balance and acidity **[7]**. The nitrogen fertilizers are currently being used only with a plant uptake efficiency of 30-50% and as a consequence it leaches into the aquatic systems nearby or are denitrified and lost to atmosphere. The economics are staggering as well, the total fertilise subsidy for the year 2008-2009 was INR 758,490 million. Production of one kg of urea would utilize 1.2l of gasoline equivalent energy. All these reasons make it very necessary to recycle fixed Nr before it degrades into nitrogen and escapes into the atmosphere and make our existence more sustainable.

Similarly, the energy involved in production of phosphorous as of 2003 was 132 million GJ per year the year in which worldwide phosphorous consumption was 31 million tons as P2O5. Phosphorous is mobilized by anthropogenic processes especially food production. Phosphorous mainly remains on land and then moves into the coastlines. Enrichment of water bodies by phosphorous would cause eutrophication which sometimes devastates the ecology of the water body. An excess of phosphorous would impact the environment negatively. Hence its recovery before discharge is very essential before it gets accumulated in a stagnant water body.

The current approach to the nutrient problem of wastewater is in general to remove N and P from the domestic and industrial wastewater streams through a sequential process of nitrification-denitrification process and precipitation. However it is now clear that, considering the above mentioned environmental and energy costs expended, N needs to be recovered and reused to remain sustainable.

Many of the micro-algae based N recovery systems developed so far require extensive cultivation practices, harvesting and processing equipment as well as expending large extents of energy. While much of the N and P lost from anaerobic digestion process is in small and much of it remains back in the wastewater particulates, simple and sustainable small scale algal systems are required. One approach towards achieving efficiency and sustainability is to grow such algae that are simple to maintain and harvest. Several studies have attempted to quantify the N-dynamics and N-removal in such algal ponds however, only a few have attempted systems which have greater ease of recovery, coupled to energy recovery/efficiency is targeted. The greater the growth rates of dispersed micro-algae (suspended growth), greater are the effort involved or energy sacrificed in algal harvest.

1.2 Naturally occurring mixed algal consortia as a treatment option:

Mixed algal consortia were chosen as the treatment option based on the following reasons:

a) Aeration: Algae provide aeration by photosynthetically providing O2 to facilitate the bacterial conversion of organic matter which corresponds to an increased efficiency in COD removal **[1, 2]**.
b) Adaptation to wastewater: As they are occur naturally in wastewater their survival would not be a problem as they would have adapted themselves to thrive in these environments over a very long period.

c) Uptake of nitrogen: Algae are known to have mechanisms for the uptake of various forms of nitrogen namely ammonia, nitrate and amino acids and these phenomena are highly variable within the algal community [3]

d) Uptake and removal of phosphorous: Algae are known to flourish in P rich waters as it is one of the main nutrients for its growth [5]. Alga also aids in the removal of phosphorous from wastewaters by adsorbtion and precipitation as growth of algae generally leads to an increase in pH [4].

e) Uptake of carbon: Quite often modes of nutrition such as mixotrophy also aid in removal of organic carbon from the wastewater and function simultaneously with bacterial degradation. Among algae, it is a process that can occur at night and therefore it results in the continuous removal of organic matter leading to a faster recovery of nutrients.

f) Ability to take up and degrade toxic organic compounds [6]: showed that the growth of some adapted algal species in the municipal solid waste leachate lead to the decrease in the toxicity of these wastewaters.

g) Ability to form clumps that either float or settle: Most of the earlier treatment methods of wastewaters have not been implementable at large scale as the energy required for harvesting of algae is prohibitive. In most cases the energetics of algal harvest decides the economy and sustainability of the overall process. Naturally clumping algae have not been studied in detail and hence their widespread implementation in wastewaters is an alternative to improve energetics of algae recovery. The property of natural settling could make true gravity settling a possibility (sans coagulants).

2. Materials and Methods:

2.1 Isolation of a stable set of mixed algal cultures: Many algal consortia were isolated from various locations in Karnataka. Such algae were maintained in minimal salt media in 500 ml plastic bottles as per standard methods.

2.2 Liquid from plug flow anaerobic digestor: Located at Centre for Sustainable Technologies, IISc, the biogas digestor was being fed fresh banana leaves on an intermittent basis as the primary feedstock. The effluent was dark brown in colour probably due to soluble lignin derivatives but was not turbid. The composition of the wastewater during different seasons is as shown in Table (1). Two replicates from each of the duplicate reactors were considered for each parameter. The liquid from the digestor was filtered through a filter of 500 μm to remove any biomass particles.

Table (1): Composition of the waste water from anaerobic digestor used for algal growth in the 4 seasons mentioned.

Leachate from anaerobic digestor	Winter	Late winter	Summer	Monsoon
COD mg/l	410±5	176±6	323±8	204±5
TKN mg/l	55.5±1.1	44.2±1.8	42.67±2.4	16±0.6
TP mg/l	8.3± 0.5	18.2±1.1	7.6±1.2	4±0.4
Alkalinity as $CaCO_3$ mg/l	1125±3	1648±10	1320±8	1577±6

2.3. Pilot studies on algal adaptability:

The procedure involved screening for adaptability and clumping. The algal consortia existing as clumps during isolation were inoculated in water from the anaerobic digestor on a wet weight basis at (measured at 100% humidity) 1g, 2g and 3g. For the consortia that existed in suspension, the inoculation was done at the concentrations of 10^4, $0.5\times(10^5)$ and 1.5×10^5 cells/ml. After a growth phase of 7 days, the consortia which were capable of forming clumps that either settle and/or float in a particular wastewater were selected.

2.4 Treatment procedure:

Operation: Batch mode: The effluent was collected from the ASTRA –CST type PFR biogas plant located at CST, IISc that was intermittently fed with banana leaves. In all, 4 sets of mixed consortia that were selected during the screening process were grown in duplicates. The reactors used for this purpose had a surface area of 0.1 m^2 each. Each tub had a working volume of 6L (height of 6 cm leading to a working volume of 6L). From preliminary runs it was determined that using a larger volume resulted in low light penetration and settled algae clumps did not rise up in response to sunlight in the mornings and therefore giving rise to lower yields. Hence the working volume was maintained at 6L for all these studies. A control used for comparison was the anaerobic digestor effluent without any external inoculation of algae. The treatment of the effluent was carried out 4 times as batch process in different seasons to check the seasonal variability (Table 2).

Figure (1): Pilot studies on adaptability

2.5 Estimation of biomass productivity:

Dry weight: This parameter was expected to give the value of harvestable dry weight by a low energy harvesting system namely by filtering out the floating clumps. At the end of the growth period in batch mode, biomass was harvested using a 50µ nylon mesh (Figure 2) and dried on an aluminium foil (Figure 3) at 90ºC till it reached a constant weight.

Table (2): Duration and season of batch test for growth of mixed consortia in leachate from anaerobic digestor.

Leachate from Anaerobic Digestor	Duration	Season
Batch 1	15/12/2010 to 21/12/2010	Winter
Batch 2	17/2/2011 to 23/2/2011	Late winter
Batch 3	12/3/2011 to 18/3/2011	Summer
Batch 4	4/8/2011 to 10/8/2011	Monsoon

Figure (2, left): Harvesting of algae using a 50 μ nylon mesh.
Figure (3): The harvested biomass smeared on aluminum foil to be dried and after drying.

2.6: Light Intensity: The treatment process involved exposure of the reactors to direct sunlight for the durations as mentioned in Table 2. During monsoon the reactors were shaded by a transparent polythene sheet at a height of 4 feet from the reactors which when tested for its shading effect with a photometer (indicated above) showed a maximum shading of 30 % at the location (inclusive of shading due to surrounding buildings). The number of sunlight hours data was obtained from the Department of Meterology at University of Agricultural Sciences, Bangalore and the intensity data was calculated from the total sunshine hours data obtained using the Angstrom-Prescott equation for the purpose of an approximate indicator of photosynthesis-$Q/Q_0 = a + b(n/N)$, where Q is the daily global radiation observed at a site, Q_0 the daily extra-terrestrial radiation (the radiation received by a horizontal plane at the top of the atmosphere), n the recorded sunshine duration (in hours), N the, day length (in hours), and a and b are empirical coefficients, estimated through a regression procedure where a=0.25 and b=0.5. The monthly average values for extraterrestrial radiation in the northern hemisphere latitudes were considered for the calculation of intensity in these months for the respective year **[8]**.

2.7 Nutrient Recovery: The biomass harvested was subject to TKN and TP analysis to estimate the percentage of N and P in each of them by the method used in soil and plant analysis **[9]**.

2.8 Statistical precision: The error bars present in the all the graphs indicate the sample standard deviation.

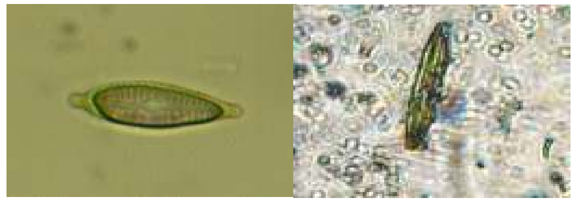
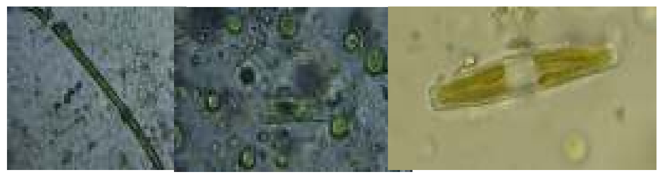
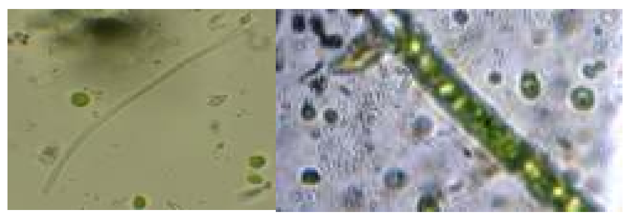
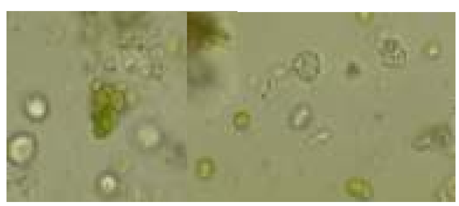
3. **Results and discussion:**

3.1. Screening and selection of algal consortia:

After an initial pilot scale screening, it was found that only the following (Table 3) mixed consortia were able to grow and form clumps in the anaerobic digestor liquid. The fourth mixed consortia was however not stable or reliable in forming clumps. Figure 4 show the algal clump formation which resulted in some of the biomass to float and a few others to settle. The Figure (3) shows mixed consortia 4 which showed very little clump formation and a large part of the algal biomass of this consortia remained largely in suspension. Though there was not much of harvestable biomass, the colour removal of the digestor liquid was high in the mixed consortia 4. In all the consortia, the part of biomass that was found floating at noon was also found to settle at night.

3.2. Biomass productivity: The four mixed consortia selected were grown in four different seasons to determine seasonal variability across the cultures as well as relative performance of species across seasons. The sunlight intensity recorded for Bangalore during the period of growth is as presented in Figure 6. The algal biomass was harvested by filtering the liquid with a 50 μ nylon mesh after a specified retention time period in batch mode. The harvestable biomass represents clumped cells among the total biomass in the growth medium. The results shown in Figure (5) are an average of duplicates. The error bars depict the population standard deviation. It is seen that the mixed consortia exhibiting the maximum productivity is different for each season and suggests a high level of adaptability among consortia. From Figure (5) we can observe that mixed consortia 1 was best suited for winter and late winter whereas for summer mixed consortia 3 is best and for monsoon mixed consortia 2 is the most suitable. By adopting this strategy the total biomass yield achievable was 1.966 kg/m^2/year assuming that each season lasts three months.

Table (3): Selected consortia after initial screening in leachate from anaerobic digestor effluent

	combinations that survived	
Mixed consortia 1 (MC1)	*Gomphonema, Navicula, Chlorella* andmembers of the Euglenophyceae	
Mixed consortia 2 (MC2)	*Anabeana, Chlorella* and *Nitzchia*	
Mixed consortia 3 (MC3)	*Ulothrix, Nodularia and Palmella* (suspected)	
Mixed consortia 4 (MC4)	*Chlorococcum and Chlorella*	

Strategy 1: Choosing the consortia according to maximum yield per season

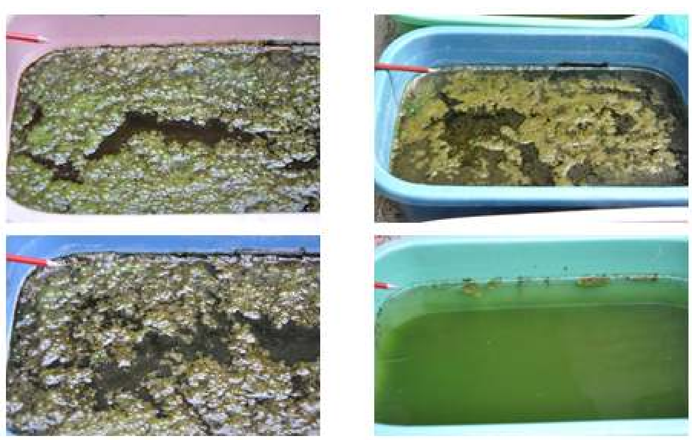


Figure (4): Ffour different consortia growing in anaerobic digestor liquid (See Table 3).

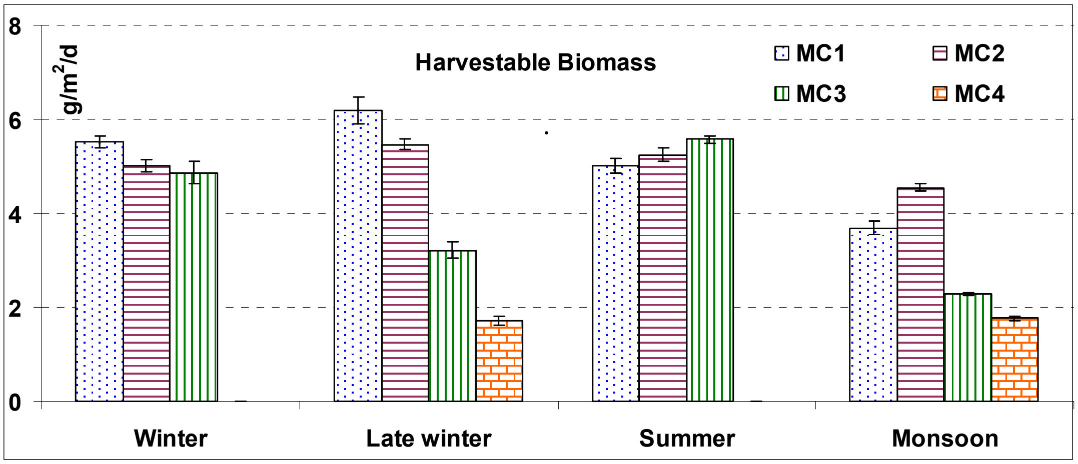


Figure (5): The harvestable biomass formed in anaerobic digestor effluent in 4 seasons across the 4 mixed consortia.

From the above results it can be said that there could be two strategies for operating the treatment process for this wastewater; one is to use the best biomass yielding consortia according to each season and second, choosing a consortia that gives the best average yield over all the seasons. Strategy 1 maximizes output whereas strategy 2 simplifies the process and maximizes the 'comfort' level to users.

Table (4): The total algal biomass productivity by adopting strategy 1 of using best consortia for each of the seasons

		Growth rate $g/m^2/d$	Biomass productivity over 3 months g/m^2
Winter	MC 1	5.515	496.35
Late winter	MC 1	6.2	558
Summer	MC 3	5.58	502.2
Monsoon	MC 2	4.55	409.5
Total yield per year=1.966 kg/m^2			

Strategy 2: Raising a single consortia throughout the year

Considering its importance as a decentralized and peri-urban/rural technology, it may be optimal to use single consortia throughout the year because of the ease of operating the wastewater treatment system and will not demand high levels of skill among the operator.

According to Table (5), if we need to choose one consortia for the entire year, there exists two choices, a) maximum annual yield and b) lowest variability or maximum guaranteed production. In order to ensure a higher yield threshold, the best choice would be mixed consortia 2, because even though its average annual biomass yield is a little lower than mixed consortia 1, it is more stable in all seasons as seen by the standard deviation. This approach would ensure a threshold level of wastewater treatment in all seasons. The total yield by this strategy would be 1.851 kg/m^2/year whereas the approach of using different mixed consortia for different seasons gives a marginally higher yield of 1.966 $kg/m^2/d$. The decision of using different mixed cultures therefore needs to be based on costs and convenience of deploying seasonally different mixed cultures.

Table (5): Average biomass over the year for all the consortia, considering four seasons of 3 months each

	Average Biomass productivity $g/m^2/d$	Std deviation	Annual Productivity kg/ m2/year
MC 1	5.105	1.06	1.86 ±0.387
MC 2	5.07	0.39	1.85±0.142
MC 3	3.98875	1.5	1.45± 0.547
MC 4	0.8725	1	0.318±0.36

3.3. Photosynthetic efficiency of mixed algal cultures:

Table (6): Photosynthetic efficiencies indicator of mixed algal consortia estimated from the total biomass harvested while growing on digestor effluent

As a %	Winter	Late winter	Summer	Monsoon
MC1	2.2	2.4	2	1.47
MC2	2	2.2	2.1	1.81
MC3	1.9	1.3	2.2	0.9
MC4	NA	0.7	NA	0.70

The photosynthetic efficiency indicator in late winter is somewhat higher than that on domestic wastewater (grown in the same season of 2011) and the reason for the same could not be established with available data. The harvestable photosynthetic efficiency was calculated on the basis of assuming 15 MJ/kg as the calorific value of the biomass and during the batch cultures, 25% shading of visible light which is used as a correction factor for the location

Although the photosynthetic efficiencies indicator (Table 6) estimated have their limitations, such as a) conversion of batch productivity to continuous, b) assumption of calorific value and c) averaging the shading effect at site, it still gives us a fairly good index for comparison. Photosynthetic efficiency of algae in wastewater is very rarely looked at **[10]** however, it is carefully studied in closed photobioreactors **[11, 12]**. The potential stimulatory effect of digestor effluent to the algae as well as its potential to be used in combination with domestic wastewater would be an interesting research area for the future.

3.4. Recovery of nutrients:

In the algal pond system there is a need to separate the different mechanisms of nutrient extraction into that which is irretrievably lost, chemically fixed and made unavailable and that which is actually recovered in algal biomass and becomes available for reuse. In this research only two components were focused, removal and recovery.

After the drying of the algae, the algal biomass was analyzed for the nutrients namely nitrogen and phosphorous. This was done in order to quantify the nutrient uptake or recovery as algal biomass (Table 7). The recovery efficiency was then calculated based on the amount of nutrient taken up and present in algae compared to the level of nutrient present in the influent in each season. It is believed that this parameter would differ in each season as the growth rates varied in each season. Sustainable wastewater treatment in the future is expected to demand recovery of nutrients from wastewater and their capture in algal biomass (in this study) and enable reuse in the agriculture or aquaculture systems later. However, conventional environmental thought processes focusing on the levels of pollution only determine removal of pollutant (N and P) from wastewater streams **[13]**. A season wise recovery of total N/P in the four different consortia across four seasons are presented in Figure 7 to 10.

3.4.1. Nitrogen recovery: From Figure (7) to (10) and Table (8) it is seen that the content of N and P in the algal biomass varies with the seasons. This could be due to change in relative population of constituent species and possibly their physiology. The consortia that showed minimum variability along with high N recovery were mixed consortia 1 and 2. The N recovery reached a maximum of 46% while the least recovery recorded was 23 % amongst mixed consortia. MC 1 was found to be best suited for the recovery of nitrogen while MC 3 was found to be the best suited consortia for recovery of phosphorous. There was a drop in nitrogen content in mixed consortia 1 biomass in the 4th batch in spite of lower TKN in the influent (this is an anomaly as N in this case is the limiting nutrient), and an increase in content of N of consortia 3 in the second batch though the nitrogen concentration is almost the same as the previous batch (Table 7). This behaviour could not be explained with available data and needs further research to ascertain the factors on which nitrogen uptake is dependent in these wastewaters. It could be that size of the constituent species is the dominating factor as size of cells determines N uptake rates **[14]**.

3.4.2. Phosphorous recovery: The recovery of phosphorous was high at 88-92% amongst the consortia studied. Hence, all of the consortia are suitable for harvest of P from the anaerobic digestor effluent as the growth medium. In control, biomass was not harvestable, hence there was no recovery. This need not represent the total phosphorous metabolically taken up by algae. It also represents the phosphorous which would have precipitated because of high alkalinity brought about due to photosynthesis. The percentage of recovery also varied on initial concentration. The recovery across species was maximum when the inlet concentration was at 15 mg/l (Figure 7 to 10).

The recovery and removal rates of phosphorous were very similar in MC1, MC2, MC3 indicating that there is low loss. Only in consortia 4 the recovery was 50 % that of removal in the two seasons when MC4 formed harvestable biomass, and in seasons when it didn't form harvestable biomass the P recovery was 0 whereas the removal was as high as 75 %.

Table (7): Nutrient recovery rates in terms of nutrient concentration from effluent across the 4 mixed consortia.

Recovery	N mg/l/d	P mg/l/d
MC 1	12.8-26	2.5-12.9
MC 2	19-24	1.9-11.7
MC 3	9.06-22.2	2.64-8.58
MC 4	5.7-7.86	2.8-9.17

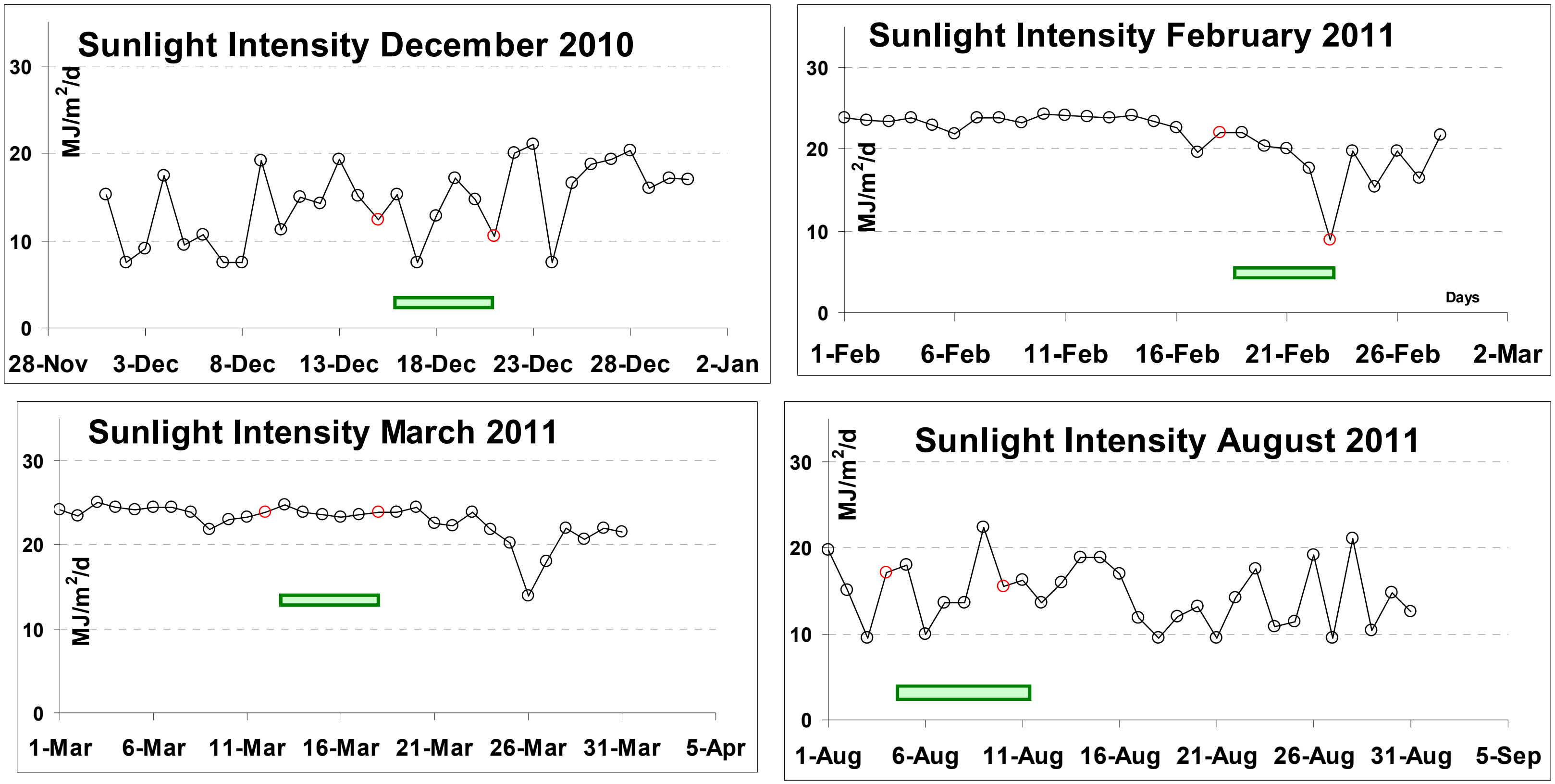


Figure (6): Sunlight intensity measured during the batch culture studies carried out for each consortium over the four specific seasons. The horizontal bar shows the sunlight intensity recorded at a nearby meteorological station coinciding with the experimental period.

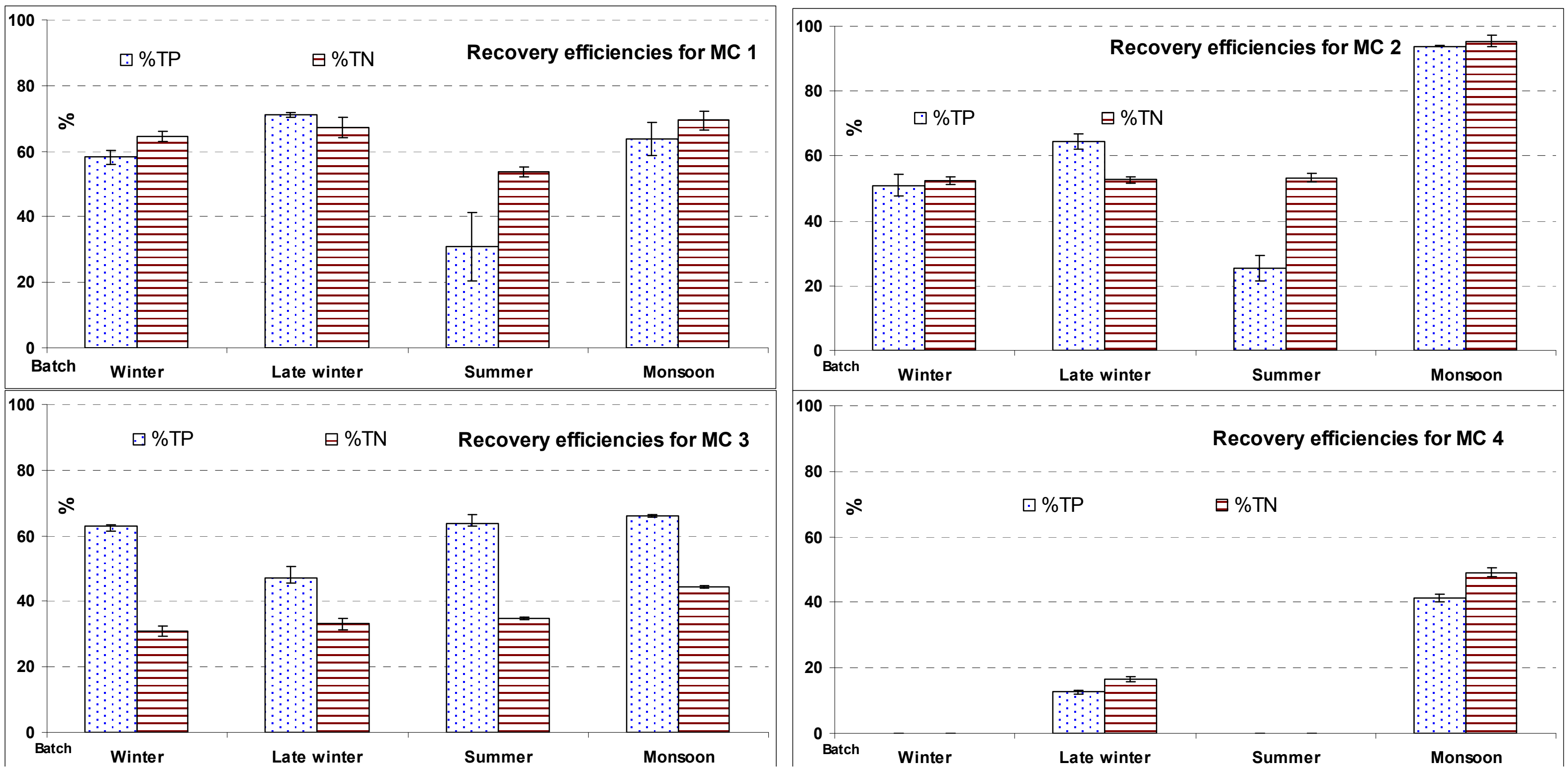


Figure (7, top left): Nutrient recovery efficiencies of mixed consortia 1. Figure (8, top right): Nutrient recovery efficiencies of mixed consortia 2. Figure (9, bottom left): Nutrient recovery efficiencies of mixed consortia 3. Figure (10, bottom right): Nutrient recovery efficiencies of mixed consortia 3.

Table (8): Nitrogen and phosphorous content in the harvested biomass.

Nitrogen content in dried algae				
	% N in MC1	% N in MC2	% N in MC3	% N in MC4
Batch 1	2.828	2.52	1.54	Na
Batch 2	2.88	2.548	2.72	2.56
Batch 3	2.744	2.6	1.596	Na
Batch 4	1.8	2.01	1.87	2.66
Phosphorous content in dried algae				
	% TP in MC1	% TP in MC2	% TP in MC3	% TP in MC4
Batch 1	0.525	0.505	0.645	Na
Batch 2	1.25	1.285	1.6	0.81
Batch 3	0.28	0.22	0.52	na
Batch 4	0.415	0.495	0.695	0.56

4. Conclusions:

The results showed that adapted mixed algal cultures could successfully be used to remove and recover N and P from anaerobic digester effluents thereby capping small nutrient losses that have been found with such biogas plants and improving the overall sustainability of the biogas systems. In this way anaerobic digester could reach zero-nutrient loss transformation systems. Total biomass harvested and N recovered are attractive to carry out further optimization for the processes. For N rich feedstock for biogas plants, such as animal and protein wastes, a substantial N fraction is expected to be found in the ammonia-N form that is potentially volatile. Removal by algae-culture and recovery in the form of algal biomass provide sustainable options that reduce the planetary overuse of N and P.

Removal (results not discussed in this paper) of N ranged between 70 - 90 % whereas the N recovery levels largely depended on the harvestability of the consortia and their N content. The N recovery ranged between an average of 30 % for mixed consortia 1 and 70 % for mixed consortia 3. The 70 % nitrogen recovery is reasonably high for an aquatic system and needs to be pursued further.

The removal of phosphorous ranged between 70 - 80 % and the recovery was once again dependant on the harvested biomass and P content in biomass. The recovery on an average was 60 %. In this kind of a system there is very low P lost by chemical and immobilization phenomena.
Increasing the inorganic carbon content in the wastewater by bubbling carbon dioxide from flue gases is expected to increase the yield of total algal biomass by almost 100 % enabling increases in the extent of nitrogen captured. However, at such high pH of 8-10 during the day, the carbon dioxide would be present as bicarbonates and the impact that it has on uptake of C needs to be studied in the context of energy and nutrient uptake efficiency.

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