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Research Article

Enhancement of Biogas Production using Pretreated Algal Biomass

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Abstract

Increasing use of fossils fuel is becoming a challenge to ozone layer depletion, climate change and other environment related issues. Algal biomass on other hand is a problem in water bodies like river, pond. Human activities have accelerated the rate and extent of eutrophication through both point-source discharges and non-point loadings of limiting nutrients, such as nitrogen and phosphorus, into aquatic ecosystems which leads to more photosynthesis by algae and their population to increase as a result. So the problems created by algal biomass and use of fossils fuel can be solved at a time if we could algal biomass as alternative source of energy.

There are studies demonstrating use of algal biomass for biodiesel production but production of biogas from biomass is not studied in detail. So we conducted this study to check the efficacy of algal biomass in producing biogas. Moreover we did some algal biomass pretreatment and compared data with untreated and differently pretreated sample. For biogas production substrate (algal biomass) is preferred to have lower protein content (20-25%), higher carbohydrate (40-45%) and lipid content (40-50%) and higher C:N ratio(20:1 - 30:1) which were also the feature of our sample.

We found that sample treated with autoclave technique contain optimal carbohydrate, protein and lipid (42.30%, 14.85%, 37.5% resp.) hence total biogas is also found highest (617.47 ml/g VS). While the untreated sample contain least carbohydrate, protein and lipid (18.78%, 3.15% 15% resp.); Thus total biogas is also found least (374.48 ml/g VS).

The findings also suggest that autoclave is the best method to disrupt algal cell compared to other techniques. Moreover pretreated algal biomass seems to produce more biogas than the untreated one as pretreatment causes cell lysis releasing the intracellular biomolecules which are converted to methane during anaerobic digestion.

Keywords: Eutrophication; Point Source Discharge; Non Point Loading; Pretreatment; C: N Ratio; Cell Lysis; Intracellular Biomolecules

Abbreviations

BMP: Biomethane Potential; BSA: Bovine Serum Albumin; C:N Ratio: Carbon to Nitrogen Ratio; D/W: Distilled Water; EJ: Exajoule($1EJ=10^{18}J$); $KNaC_4H_4O_6.H_2O$: Potassium Sodium Tartrate Monohydrate; LCFA: Long Chain Fatty Acid; Nm: Nanometer; TCA: Trichloro Acetic Acid; TS: Total Solid; TBMP: Theoretical Biomethane Potential; TVS: Total Volatile Solid; TFS: Total Fixed Solid

Introduction

Nowadays, we are looking for the pathway to a sustainable energy supply with high reduction in greenhouse gas emissions and less dependency on fossil fuel. Biomass is expected to be a good solution for energy purpose [1].

Graph 1: Pie chart showing worldwide consumption of energy source [2].

In past decades' energy crisis causes huge decrease of unstainable resources like fossil fuels. The excess use of fossil fuel led to excess release of Carbon dioxide increasing global warming. High demand of energy is a matter of concern and thus, must give attention to production of sustainable source of energy like Biofuels [3].

Biofuels are gaseous or liquid transportation or heating fuels derived from biological sources, such as grains, sugar crops, starch, cellulosic materials, and organic waste [4].

Biogas is one of the type of biofuels which can be use as alternative source for the fossil fuel and is produced by anaerobic decomposition or thermochemical conversion of biomass. Biogas is composed mostly of methane and carbon dixide. Methane content of raw biogas vary from 40-60%, with Carbon dioxide making up most of the remainder along with small amount of water vapor and other gases [5].

Microalgal biomass can be promising approach for enhancement of biogas production. Microalgal biomass is the amount of algae in the water body in a given time. Harvesting of microalgal biomass is done by separating algal biomass from water. Algae-based biogas is technically and economically viable and cost competitive, require no additional lands, require minimal water use, and mitigate atmospheric CO_2 [6].

Theoretical methane yield of microalgae is dependent on lipid, carbohydrate, protein, and lipid of microalgal biomass. In general protein accounts for 43-70% of dry biomass followed by carbohydrate (33-64%) and lipid (20-70%).

Pretreatment techniques were pointed out as necessary for microalgal cell disruption and biogas production by Chen and Ostwald (1998). The efficiency of pretreatment method on biogas production depends on different characteristics of microalgae, i.e., toughness and structure of cell wall, and micro molecular composition of cells [7]. The complex cell wall structure of micro algae provide resistance to biological attack thus, species without cell wall (e.g. *Dunaliella* sp.) or containing a glycoprotein cell wall (e.g. *Euglena* sp.) showed higher methane yield then those with a more complex cell wall (e.g. *Chlorella* sp.) [8].

Pretreatment can be done by physical operation (e.g. ultrasonification, autoclave, beadmill) or chemical treatment (e.g. salt, acid) or biological treatment (enzymatic) [9].

Pretreatment of algal biomass allows us to: fractionate biomass in a way that makes it easier to carry out subsequent process steps, improve uniformity and hydrophilic characteristics, increase chemical contact and reaction, efficient biogas production, decrease hydrolysis time [4].

Bio methane potential is defined as maximum volume of methane produced per gram of volatile solid substrate. BMP indicates biodegradability of a substrate and its potential to produce methane via anaerobic digestion [10].

Research hypothesis

The use of pretreatment of microalgal biomass is supposed to increase biogas production.

Null hypothesis (95% C.I)

There will be no biogas production without pretreatment of microalgal biomass.

Alternative hypothesis (95% C.I)

There will significant increase in biogas production in one of different pretreatment techniques of microalgal biomass.

Research objective

General objective

To evaluate BMP using pretreated algal biomass.

Specific objective

- To estimate total carbohydrate, total protein, total lipid, C/N ratio, ash content, total solid of algal biomass
- To compare the parameter in different pretreated biomass
- To understand role of algal biomass in enhancement of biogas production
- To make use of algal biomass for biogas production.

Research scope

- To make use of algal biomass which are causing problems in water bodies
- To search alternative of fossils fuels (through biofuels - specifically in biogas)
- To increase the biogas production through pretreatment of substrate
- To compare different methods of pretreatment and comparing their biogas producing efficiency.

Materials required

- Autoclave
- Beaker
- Micropipette
- Weighing machine
- Hot air oven
- Measuring cylinder
- Ultrasonicator
- pH meter
- Water bath
- Centrifuge
- Vortex
- Glass bead (0.4-0.6 mm)
- Conical flask
- Behive shelf
- Rubber tube
- Glass jar
- Gas cylinder
- Muffle furnace
- Crucible
- Vaccum drier
- Spectrophotometer
- CHN analyzer
- Test tube
- Distilled water
- Nacl
- Thermostable cellulase
- $CHCl_3$
- CH3OH
- TCA
- Na_2CO_3
- $CuSO_4.5H_2O$
- $KNaC_4H_4O_6.4H_2O$
- Folin cicolfeu phenol
- H_2SO_4
- $C_{14}H_{10}O$
- $C_6H_{12}O6$
- BSA
- KOH
- C_2H_5OH (absolute).

Methods

Sample collection

Pure and dried culture of *Chlorella sorokiniana* is provided by algal research laboratory, Department of Biotechnology from Kathmandu University, Nepal.

Preliminary analysis

Preliminary analysis of sample and inoculums was done following protocol given by VDI 4630 (VDI, 2006).

Total solids

- At first weight of crucible was taken as W dish after heating 105 degree Celsius for 1 hrs. in muffle furnace.
- 25 gm of algal biomass was taken in crucible; weight was measured as W sample.
- Then, crucible containing sample was heated at 105 degrees Celsius for 12 hrs.

- Then, weight of crucible containing sample was measured as W total.

Calculation

% Total solid = (W total - W dish)/(W sample - W dish) * 100%

Note: W total = Weight of dry residue and dish (g)

W dish = Weight of dish (g)

W sample = Weight of wet sample and dish (g).

Fixed solids and volatile solid

- Crucible containing dried residue obtained from above step was taken
- Then, it was ignited in muffle furnace at 550 degrees Celsius for 2 hrs.
- The crucible was then, cooled in desiccator to balance the temperature.
- Final weight of crucible as W volatile.

Calculation

% Volatile solid = (W total - W volatile)/(W total - W dish) * 100%

% Fixed solid = (W volatile - W dish)/(W total- W dish) * 100%

Note: W total = Weight of dry residue and dish (g)

W volatile = Weight of residue and dish after ignition (g)

W dish = Weight of dish (g).

C: N ratio

- The microalgae biomass of isolate "GNP" was sent to Nepal Environmental and Scientific Services Pvt. Ltd for C:N analysis.
- Total Organic Carbon was analyzed as per Walkley and Black, Anderson J.M. and Ingram, J.S.I/USDA/FAO Bulletin No. 19.
- The nitrogen was determined by Microkjeldahl Digestion, FAO Bulletin no. 19 method.

Pretreatment of Micro algal biomass

Autoclave

- 5g of algal sample was taken.
- Then, sample was diluted in 100 ml of distilled water.
- Autoclave was maintained at 121 degrees Celsius, 15 lbs. pressure for 20 min.
- Water was evaporated in Hot air oven at 66 degrees Celsius for 6 hrs.
- Dried sample was obtained for further analysis.

Osmotic shock

- 5g algal sample was taken and diluted in 12.5 ml distilled water.
- Then, the sample was mixed with 5 ml of 10% Nacl.
- The mixture was then, vortexed for 2 min.
- The contents were further incubated for 48 hours for room temperature.
- Water was evaporated in Hot air oven at 66 degrees Celsius for 6 hrs.
- Dried sample was obtained for further analysis.

Bead mill

- 5g of algal biomass sample was taken.
- 150 ml of distilled water was taken in beaker and algal biomass was added.
- Further glass bead (0.4-0.6 mm) was added in the beaker in the ratio of 3:1 (biomass: glass bead).
- Then, sample was vortexed for 1060 cycle per minute for 30 minutes.
- Water was evaporated in Hot air oven at 66 degrees Celsius for 6 hrs.
- Dried sample was obtained for further analysis.

Ultrasonification

- Algal biomass 0.5g was mixed with 15 ml of sterile distilled water (standard 40g/l).
- Then, sample was sonicated at 24 kHz for 30 minute [7,11]
- Water was evaporated in Hot air oven at 66 degrees Celsius for 6 hrs.
- Dried sample was obtained for further analysis.

Enzymatic treatment

- 5 gm algal sample is taken in 150 ml DW.
- 0.1 M sodium citrate buffer (PH 5.5) is prepared in 100 ml beaker.
- Then, Cellulase enzyme at concentration 5 mg/L is added.

- Then, enzymatic hydrolysis is conducted at 37 degrees Celsius for 2 hrs.
- Cellulase is inactivated by heating at 100 degrees Celsius for 10 minute and then, sample is dried.

Analysis of pretreated and untreated algal biomass

Carbohydrate content measurement

For standard glucose curve

Stock Solution(µl)	0	10	50	100	200	300	500	1000
Distilled water(µl)	1000	990	950	900	800	700	500	0
Final Concentration(µg/µl)	0	0.1	0.5	1	2	3	5	10

Table 1: Different glucose concentrations sample to prepare standard glucose curve.

- 0.4 ml of the final solutions of Glucose was mixed and vortexed with 0.8 ml of prechilled 75% H_2SO_4 solution in test tubes.
- To the mixture, 1.6 ml of freshly prepared anthrone reagent was added.
- The samples are then, boiled at 100°C for 15 minutes.
- The sample were cooled to room temperature and absorbance read at 578 nm.

Carbohydrate content measurement

- 10 mg of pretreated dried microalgae sample was resuspended in 0.2 ml of distilled water.
- The sample was heated in 0.4 ml of 40% KOH at 90°C for one hour.
- The sample was then, cooled to room temperature.
- 1.2 ml of cold absolute ethanol was then, added to the sample and stored in fridge at -20°C overnight.
- After that, the sample was centrifuged at 3000 rpm for 10 minutes and the supernatant was discarded.
- The pellet was resuspended in 1.5 ml of distilled water.
- 0.4 ml of the resuspended pellet was mixed and vortexed with 0.8 ml of prechilled 75% H_2SO_4 solution in a test tube.
- To the mixture, 1.6 ml of freshly prepared anthrone reagent was added.
- The sample was then boiled at 100°C for 15 minutes.
- The sample was cooled to room temperature.
- The sample was then diluted by 10 times with 75% H_2SO_4 and the absorbance was read at 578 nm.

Protein content measurement

For standard BSA curve

Stock Solution (µl)	0	10	50	100	200	300
Distilled water (µl)	1000	990	950	900	800	700
Final Concentration (µg/µl)	0	0.1	0.5	1	2	3

Table 2: Different BSA concentrations sample to prepare standard BSA curve.

- 1 ml of final BSA solution was taken in Eppendorf tube.
- 1.4 ml of lowry reagent was added to the tube and vortexed for 3 minute and incubated for 20 minute in dark.
- Then, 0.2 ml of folin reagent was added to the tube and vortexed for 3 minute and incubated for 30 minute in dark.
- Absorbance was then, read at 600 nm.

Protein content measurement

Protein estimation

- 5 mg algal biomass sample was taken.
- Then, the sample was vortexed in distilled water in 0.2 ml 24% (w/v) TCA.

- Then, the sample was incubated at 95 degree Celsius for 15 min.
- TCA precipitation occured which is cool down to room temperature.
- Then, 0.6 ml water was added to the precipitate and centrifuged the sample at 15000 rpm for 20 minute at 4 degree Celsius.
- Supernatant after centrifugation was discarded while pellet was resuspended in 0.5 ml lowry reagent D.
- This alkaline suspension was incubated at 55 degree celsius for 3 hour and centrifuged 15000g for 20 minute at room temperature.
- Pellet from centrifuged sample was discarded while supernatant was retained which contained protein.

Protein quantification

- 50 microliter of protein extract was added to 1.5 ml Eppendorf tube in triplicate followed by 950 microliter of lowry reagent D followed by immediate mixing.
- The sample was incubated for 10 minute in room temperature.
- Next 0.1 ml of dilute Folin ciocalteu phenol reagent was added to each tube and vortex immediately.
- After 30 minute at room temperature absorbance of each sample was taken at 600 nm.

Lipid content measurement

Lipid content was measured following [12].

- Weight of dried Eppendorf tube was taken as w1.
- 20 mg lysed algal biomass was taken in Eppendorf tube with 80 µL DW and 300 µL of chloroform-methanol (1:2).
- Sample was then, vortexed for 2 min.
- Again 100 microliter of chloroform was added and again vortexed for 30 second.
- Then, 100 microliter of distilled water was added to create 2 phase system and vortexed for 30 second.
- Tubes was then, centrifuged at 2500g for 6 minute
- The clear aqueous phase was discarded and chloroform phase was recovered and residue was re- extracted with 100microliter chloroform and centrifuged as above.
- The chloroform phase (first portion) was extracted and kept in a Eppendorf tube of known weight.
- Eppendorf tube containing chloroform phase was dried at 62 degree Celsius in hot air oven and then, its weight was measured as w2.
- Lipid content was calculated.

BMP measurement

Preparation of test medium

Test medium was prepared according to VDI 4630 and ISO Standard 11734 (ISO11734, 1995).

Chemicals	Amount (g)
Anhydrous potassium dihydrogen phosphate (KH_2PO_4)	0.27
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	1.12
Ammonium Chloride (NH_4Cl)	0.53
Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)	0.075
Magnesium chloride Hexahydrate ($MgCl_2 \cdot 6H_2O$)	0.10
Sodium sulphide Nonahydrate ($Na_2S \cdot 9H_2O$)	0.1
Iron (II) chloride Tetrahydrate ($FeCl_2 \cdot 4H_2O$)	0.02

Table 3: Consitutents of test medium.

The constituents were added to 1 liter distilled water containing less than 1 mg/L dissolved oxygen.

Anaerobic diegstion

- Pretreated algal biomass along with untreated algal biomass was taken as substrate for biogas production.
- Similarly, incolum was collected from local biogas digester.
- Then, volatile solid both substrate and inoclum was measured.
- For each test, 250 ml glass bottle (reactor) were prepared.
- 13.5 ml test medium was added to glass bottle.
- In addition to test medium inoclum and substrate with ratio 2:1 (VS basis) was added and the final volume was made 60 ml.
- Waterbath was maintained at thermophlic temprature of 55^0C.
- The glass bottle containing the mixture of testmedium, incolcum and substrate was placed in water bath and externaly connected to 50 ml measuring cylinder containing 0.5 M HCL solution in reservior.
- Then, Degassing was done for 2 days by leaving glass bottle without collecting gas.

Gas volume measurement

For gas volume measurement we followed liquid displacement method.Similarly for biogas production we followed Hansen's protocol [13].

- A measuring cylinder was filled with 0.5M HCl which opened to a container containing same liquid.
- The headspace of the reactor was connected with a pipe which opened to the measuring cylinder.
- The volume of gas collected was noted after shifting the cylinder to adjust the level of the liquid inside it with that in the container outside such that the pressure inside the cylinder is equivalent to that outside.
- Then, potassium hydroxide was added to absorb CO_2 content of the biomass which gave the remaining CH_4 amount.
- The height was again adjusted according to the shifted volume and the CH_4 amount was noted down.

Results

Preliminary analysis of algal biomass and inoculum

Total solid

The total solid was determined by heating the crucible containing sample in muffle furnace for 105 degrees Celsius for 12 hrs. Total solid in *Chlorella sorokiniana* is found to be 55-66% [14]. Total solid in inoculum (cow dung) is found around 16.28% [15].

Parameters	Algal biomass	Inoculum
Total solid	75.15%	3.73%

Table 4: Result of total solid of algal biomass and inoculum.

Total volatile and fixed solid

The volatile solid was determined by igniting sample in muffle furnace at 550 degrees Celsius for 2 hrs. Total volatile solid in *Chlorella sorokiniana* is found to be around 2.81 ± 0.12 gm/L [16]. Total volatile solid in Inoculum (cow dung) is found around 69% [17].

Parameters	Algal biomass	Inoculum
Volatile solid	12.55%	79.54%
Fixed solid	12.5%	16.73%

Table 5: Result of volatile solid and fixed solid of algal biomass and inoculum.

Figure 1: Heating of sample in muffle furnace.

Figure 2: Weight of crucible containing sample.

C: N ratio

The C: N ratio of *Chlorella sorokiniana* is found around 7:1. For C: N ratio estimation sample was sent to Nepal Environmental and Scientific Services Pvt. Ltd. The C: N ratio was found to be 9:1 of algal biomass.

Analysis of pretreated and untreated algal biomass

Lipid

The lipid analysis was done using [12] using two phase extraction process with use of chloroform, methanol, distilled

water. The lipid content of microalgae is usually in the range of 20-50% of the cell dry weight, and can be as high as 80% under certain conditions [18]. The lipid content of *chlorella sorokiniana* estimates around 18-22% lipid of dried biomass [19].

Results of lipid analysis are shown in table 6.

Methods	Lipid content (%)
Autoclave	37.5
Bead mill	24
Enzymatic	25
Osmotic shock	28.5
Ultrasound	24
Untreated sample	15

Table 6: Lipid analysis of pretreated and untreated algal biomass.

Graph 2: Comparison of Lipid% in untreated and different pretreated algal sample.

Figure 3: Formation of 2 phase system during lipid analaysis.

Figure 4: Pellets after discarding supernatant.

Carbohydrate

The carbohydrate content was measured spectrophotometrically using anthrone- sulphuric method [20]. In this method different reagent like anthrone, 40% KOH, ethanol, 75% H_2SO_4. Carbohydrate content of algal biomass of *chlorella sorokiniana* ranges about 20-45% of its dry weight [21].

The standard Glucose solutions (0, 0.1, 0.5 and 1 mg/l) were used to create a standard curve. The 0 mg/ ml glucose sample was used to prepare the reagent blank. From the absorbance of standard glucose solutions at 578 nm, a standard curve was prepared as.

Graph 3: Standard curve of glucose.

Figure 5: Color change in glucose standard solution.

Results of Carbohydrate analysis is shown in table 7.

Methods	Carbohydrate content (%)
Autoclave	42.30 ± 0.32
Bead mill	33.67± 0.65
Enzymatic	31.40 ± 1.30
Osmotic shock	19.99 ± 0.588
Ultrasonification	25.63 ± 0.13
Untreated sample	18.78 ± 0.21

Table 7: Carbohydrate analysis of pretreated and untreated algal biomass.

Graph 4: Comparison of carbohydrate % in untreated and different pretreated algal sample.

Figure 6: Incubating samples at 950C.

Figure 7: Pellets retained after centrifugation.

Protein

There are two commonly used methods for spectrophotometric quantification of microalgae which are "Lowry Method" and "Bradford Assay". The quantity of protein estimated with Bradford assay is found to be lower than that with Lowry Method [22]. Lowry method was preferred for this thesis work. The protein content of *chlorella sorokiniana* estimates around 35-50% of dried biomass (Kobayashi., *et al.* 2013).

The standard BSA solutions (0-3 µg/µl) were used to create a standard curve. The 0 µg/µl BSA sample was used to prepare the reagent blank. From the absorbance of BSA solutions at 600 nm, a standard curve was prepared as:

Graph 5: Standard curve of BSA.

Figure 8: Color change in BSA standard solution.

Results of Protein analysis is shown in table 8.

Methods	Protein content (%)
Autoclave	14.85 ± 0.06
Bead mill	13.28 ± 0.05
Enzymatic	12.94 ± 0.052
Osmotic shock	13.9 ± 0.056
Ultrasonification	18.15 ± 0.073
Untreated sample	3.15 ± 0.09

Table 8: Protein analysis of pretreated and untreated algal biomass.

Graph 6: Comparison of protein% in untreated and different pretreated algal sample.

Figure 9: Reagents used for protein estimation.

Figure 10: Vortexing of sample.

Figure 11: Incubation of sample.

Bio methane/Biogas potential

The Bio methane/Biogas potential was measured using Hansen method followed by liquid displacement method. The Bio methane/Biogas potential of *Chlorella sorokiniana* was found to around 700-800 litre/kg VS basis [11].

Theoretical biogas yield was determined according to [23] as:

TBMP = (lipid * 1014 + protein * 496 + Carbohydrate * 415 + lignin * 727) * 0.001

The value of TBMP is represented as kg/ VS and lipid, protein, carbohydrate and lignin as g(kg/vs).

Methods	TBMP (ml/g VS)
Autoclave	497.26
Bead mill	254.68
Enzymatic	353.91
Osmotic shock	348.46
Ultrasonification	383.45
Untreated	193.48

Table 9: Theoretical biogas yield of untreated and different pretreated algal biomass.

Methods	Gas produced	Volume (ml/g VS)
Inoculum (control)	Biogas	103.6 ± 15.18
Untreated	Biogas	374.48 ± 15.09
Autoclave	Biogas	617.47 ± 32.34
Bead mill	Biogas	516.36 ± 31.59
Enzymatic Osmotic shock	Biogas Biogas	425.02 ± 15.55 399.75 ± 10.1
Ultrasonification	Biogas	475.625 ± 31.64

Table 10: Biogas potential of untreated and different pretreated algal biomass.

Figure 12: Biogas digester.

Figure 13: Overall setup of Biogas potential measurement.

Figure 14: Gas collected by liquid displacement method.

Inoculum (control)

The total gas produced from inoculum is 401 ml and on basis of volatile solid it is found to be 103.6 ± 15.18 ml/g VS.

Graph 7: Total biogas produced from Inoculum only.

Untreated

Graph 8: Total biogas produced from untreated algal sample.

The total gas produce from untreated algal sample is 864 ml and on basis of volatiles solid is 374.48 ± 15.09.

Bead mill

The total gas produced from Bead mill is 1019 ml and on basis of volatile solid it is found to be 516.36 ± 31.59.

Graph 9: Total biogas produced from sample treated with bead mill technique.

Autoclave

Graph 10: Total biogas produced from sample treated with autoclave as pretreatment technique.

The total gas produced from Autoclave was 1080 ml and on basis of volatile solid it was found to be 617.47 ± 32.34 ml/g VS.

Enzymatic

Graph 11: Total biogas produced from sample treated with enzyme.

The total gas produced from enzymatic pretreatment is 925 ml and on basis of volatile solid it is found to be 425.02 ± 15.55 ml/g VS.

Osmotic shock

The total gas produced from enzymatic pretreatment is 914 ml and on basis of volatile solid it is found to be 399.75 ± 10.1 ml/g VS.

Ultrasonification

The total gas produced from Ultrasonication pretreatment is 987 ml and on volatile solid basis is found to be 475.625 ± 31.64 ml/g VS.

Graph 12: Total biogas produced from sample treated with Osmotic Shock.

Graph 13: Total biogas produced from sample treated with Ultrasonification.

Graph 14: Comparison of total biogas of untreated and pretreated sample.

Discussion

Preliminary analysis of algal biomass and inoculum

Total solid

On performing the preliminary analysis of algal biomass and inoculum total solid of algal biomass was found around 75.15% and inoculum 3.73%. Our analysis shows similar result with research conducted as value of total solid range from 50-65% for algal biomass and total solid for inoculums was found less than research done by others this might be due to presence of high water content in inoculum than solid content.

Total volatile solid and fixed solid

Total Volatile solid of algal biomass was found around 12.35% and inoculum around 79.54%. Our analysis shows similar result with research conducted as value of total volatile solid range from.

C: N ratio

The C: N ratio of algal biomass was found to be 9:1. The standard C:N ratio was reported around 7:1 for *Chlorella sorokiniana* (Kumar., *et al.* 2014). The optimum C: N ratio for biogas production is found to be in the range of 16: 1 - 25: 1 [24]. The substrate which has low C: N ratio contains relatively higher concentrations of ammonia, which exceeds the optimum amount that is required for biogas production and can inhibit the anaerobic digestion process. Moreover, the substrate with a very high C: N ratio is found to have poor buffering capacity and possibility of accumulation of high volatile fatty acids during the digestion process.

Since, our sample (algal biomass) has lower C: N ratio it is recommended to use ash, paper waste, agricultural waste to increase the carbon content

Analysis of pretreated and untreated algal biomass

Lipid

The lipid content of untreated algal biomass was found around 15% and Pretreated Sample i.e. Autoclave, Bead mill, Enzymatic, Osmotic shock, Ultrasound was found around 37.5%, 24%, 25%, 28.5%, and 24% respectively. Our analysis shows similar result with research conducted as lipid content of *Chlorella sorokiniana* estimates around 18-22% lipid of dried biomass [19]. From our thesis work lipid content was found to be higher with algal sample treated with autoclave. During this pretreatment technique microalgal cell ruptures releasing intracellular component due to

high thermal stress. For Biogas production higher lipid content is favorable as long chain fatty acids are converted to methane. Optimum lipid concentration for biogas production is around 12.8-19.7%, when the lipid content is higher about 59.3% gas production decreases sharply [25].

Least lipid content was found least in untreated algal sample as intracellular component are not released.

Carbohydrate

The Carbohydrate content of untreated algal biomass was found around 18.78 ± 0.21 and Pretreated Sample i.e. Autoclave, Bead mill, Enzymatic, Osmotic shock, Ultrasonification was found around 42.30 ± 0.32%, 33.67 ± 1.30%, 31.40 ± 0.588%, 19.99 ± 0.13%, 25.63 ± 0.13%, respectively. Our result shows alike result with research conducted as Carbohydrate content of algal biomass of *chlorella sorokiniana* ranges about 20-45% of its dry weight [21]. From our research work Carbohydrate content was found to be higher with algal sample treated with autoclave. During this pretreatment technique microalgal cell ruptures releasing intracellular component due to high thermal stress.

Protein

The Protein content of untreated algal biomass was found around and Pretreated Sample i.e. Autoclave, Bead mill, Enzymatic, Osmotic shock, Ultrasound was found around 14.85 ± 0.06%, 13.28 ± 0.05%, 12.94 ± 0.052%, 13.9 ± 0.056%, 18.15 ± 0.073% respectively. The protein content of *chlorella sorokiniana* estimates around 35-50% of dried biomass [26].

Protein content was found to be lower than research conducted by [26] because our sample is cultured in such a way to produce more carbohydrate. For biogas production lower protein content is preferred as ammonia inhibits methane production. So, our algal biomass is favorable to use as a substrate for biogas production.

Bio methane/Bio gas potential

From the research conducted bio gas potential of untreated dried biomass of *Chlorella Sorokiniana* is found to be 275 ml g^{-1} VS [27]. From our thesis work biogas potential of dried biomass of *Chlorella Sorokiniana* was found to be 374.48 ± 15.09 ml g^{-1} VS which is little bit higher than research done by [27], this is because our algal biomass sample was optimized to produce more carbohydrate.

Similarly, for pretreated algal biomass biogas potential seems to be higher than untreated biomass i.e. 617.47 ± 32.34 ml g^{-1} VS, 516.36 ± 31.59 ml g^{-1} VS, 425.02 ± 15.55 ml g^{-1} VS, 399.75 ± 10.1 ml g^{-1} VS, 475.625 ± 31.64 ml g^{-1} VS for autoclave, beadmill, enzymatic, Osmotic shock, Ultar sonification respectively.

Among the different types of pretreatment, thermal pretreatment is considered efficient in the case of Microalgae as reported by [13]. From our research work also thermal pretreatment (autoclave) shows similar result as done by [13]. Similarly, sample treated with autoclave give highest biogas yield compare to other pretreatment techniques. Lower yield in untreated sample is because cells are not disrupted in untreated sample so the intracellular components are not released; Hence biogas potential is found lower.

From this thesis work it is proved that pretreatment of algal biomass is necessary to enhance the biogas production.

Conclusion

The microalgal species isolated was analyzed for the production of biogas as a renewable sustainable source of energy. Four different parameters were analyzed for checking the efficiency of algal biomass for biogas production. Further different pretreatment of algal biomass was carried which also seems to increase the biogas production than untreated sample. The best strain was used for the production of biogas as a source of energy.

This thesis provided us the opportunity to learn about the basic principles of research, research methodology and contribute something for the development of sustainable energy sources. The world faces a major problem of fuel crisis and in this scenario microalgae can prove as an excellent alternative source for generation of biomass energy.

Nepal's tropical climate, huge water resources and diversity can be utilized in a state of the art processes making algal biomass energy economically feasible for this country. The finding of this research shows that microalgae possess potential for biomass energy generation due to their productivity and biomass generation in large quantity in short interval.

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