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Biodiesel Production from Wet Microalgae Biomass through Direct Transesterification by Conventional and Microwave Radiation Method

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Abstract

The present work investigates the effect of methanolic HCl on fatty acid methyl ester (FAME) yield during in-situ (direct) transesterification of highly wet microalgae *Neochloris oleoabundans* biomass. The direct transesterification was conducted in tightly sealed glass tube by mixing methanolic HCl, solvent and wet algal biomass and maximum FAME yield (90%) was obtained at 90^0C. The effect of different reaction parameters were analyzed and optimized such as methanolic HCl %, solvent amount, temperature and time for different ranges. The moisture content above 20% significantly decreases the biodiesel yield. However with increasing the amount of methanolic HCl in wet algae, more FAME was recovered. Conventional direct transesterification method was compared with microwave assisted transesterification and it was found that biodiesel yield was enhanced to 90% with microwave method. So, microwave assisted transesterification is one of the best technique to produce biodiesel. Finally, the FAME was quantified by GCMS and it was found that *Neochloris oleoabundans* is a potential feedstock for production of biodiesel through direct transesterification having all the major fatty acid components.

Keywords: FAME; In-situ transesterification; methanolic HCl; microalgae; optimization; *Neochloris oleoabundans*.

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INTRODUCTION

The microalgae is a potential feedstock for biodiesel production which replaces the petro-diesel [1]. Microalgae produce oil, proteins and polysaccharides by photosynthesis [2]. Some microalgal species are rich in oil as compared to plants and it converts water and CO_2 to macromolecules [3]. Till now lot of research has been focused on vegetable oil but now efforts are devoted to produce biodiesel from microalgae [4]. However due to numerous technical problems and high energy requirement for extraction of oil it becomes un-feasible to commercialize biodiesel production [5]. The conventional biodiesel production technique is a complex process that involves cell disruption followed by collection of oil using different solvents and then transesterification of oil [6]. So oil extraction is high energy consuming because microalgal cell walls are firm and thick which are difficult to break. Study shows that when the algal biomass is wet it negatively influence the biodiesel yield, so Hidalgo (2013) suggested direct transesterification method for oil extraction from wet algal biomass that involves two steps in a single reaction that is lipid extraction and conversion to biodiesel simultaneously [7]. Here one-step (direct) transesterification incorporates both esterification and transesterification of free fatty acids and triglycerides respectively. Thus simplifying the biodiesel production process by eliminating the oil extraction step that includes lot of oil loss.

In direct transesterifcation process FAME yield highly depends upon presence of catalyst [8]. So numerous studies have been conducted on different catalysts such as enzymes, homogenous and solid catalysts [9]. Presence of moisture negatively affect the FAME yield when alkaline catalysts are used because of saponification [10]. Therefore more work is conducted on acid catalysts such as BF_3 and H_3PO_4 [11]. Biodiesel production by in – situ transesterification from dry microalgae using H_2SO_4 as a catalyst is getting more attention [12] because dry algae reacts with methanol and H_2SO_4 where methanol solves the purpose of both solvent and esterification reagent. However, very less work was conducted on wet microalgae. Velasquez – orta et al., (2013) used H_2SO_4 as a catalyst for different moisture content in algal biomass and obtained maximum FAME yield of 73% [13].

In this research work a best feedstock rich in lipid content has been identified and biodiesel production process was optimized and effective method for transesterification was found to be microwave assisted method. Effect of methanolic HCl was not addressed yet on wet microalgae. The present work examined the efficiency of methanolic HCl in in – situ transesterification of wet algae which act as both catalyst and solvent. Furthermore effect of methanolic HCl was addressed when moisture content in the algae biomass was very high which was not investigated till now for wet microalgae. Finally impact of variation of methanol, reaction temperature and methanolic HCl was explored and optimized to get high yield.

MATERIALS AND METHODS

MATERIALS

In the present study, microalgae *Neochloris oleoabundans* (UTEX 1185) was chosen and obtained from Texas University Austin, USA. Soil extract medium was purchased from university of Texas. Methanolic HCl was prepared using acetyl chloride and methanol in chemistry Lab Delhi technological university, Delhi, India. Acetyl chloride and methanol HPLC grade, ≥99.9% was obtained from Sigma-Aldrich. Chloroform was procured from Fisher Scientific.

ALGAE CULTIVATION

Microalgae *Neochloris oleoabundans* was further grown in a Bristol medium and glucose 9g/L is supplemented as a carbon source to grow heterotrophically. Bristol medium composition is shown in Table 1. The temperature of the media was kept at 29^0C and media was manually shaked thrice a day. Finally after 10 days the algae was harvested by sedimentation and centrifugation at 3000 rpm for 15 min. Centrifuged biomass was further used for direct transesterification.

Table 1 Bristol medium components.

Bristol medium	**$NaNO_3$**	**$CaCl_2.2H_2O$**	**$MgSO_4.7H_2O$**	**K_2HPO_4**	**KH_2PO_4**	**NaCl**
(in mM)	2.94	0.17	0.3	0.43	1.29	0.43

MICROWAVE ASSISTED DIRECT TRANSESTERIFICATION

0.200 gm centrifuged algal biomass was mixed in flask with 10 ml distilled water. Then this mixture was further heated by a microwave up to 5 min at 900W with 5 sec rest period after every 20 sec heating period. Then 3 ml of 8% methanolic HCl and 7.5 ml solvent was added. This mixture was heated at 90^0C for 30 min. The upper layer containing lipid was pipetted out and weighed.

DIRECT TRANSESTERIFICATION OF WET ALGAE.

For direct transesterification Lepage & Roy (1986) method was adopted and modified [14]. Solution was made by adding solvent (methanol: chloroform 3:1) and wet algae biomass with internal standard (C:17). Further in the above solution 2 to 12% methanolic HCl was added in the glass tube and sealed with cap. This Methanolic HCl was prepared according to Sukhija and Palmquist, (1988) [15]. For 5% methanolic HCl, 10 ml of acetyl chloride was added drop by drop to 100 ml methanol. Glass tubes were placed in water bath at 90^0C for 80 minute to complete the reaction. After that samples were taken out and cooled to room temperature. To neutralize the reaction 0.9% Nacl was added. FAMEs were collected by adding 3 ml of hexane to solution and heating at 90^0C for 20 min. Finally solution was centrifuged at 2000 rpm

to separate biomass. Upper layer containing FAME was collected and quantified by GCMS. Biodiesel yield depends upon various parameters such as moisture content in algae, reaction temperature and time. Table 2 shows the optimization parameter range. For measuring the effect of different parameters microalgae biomass with constant moisture content 20% was taken. All the work was done in triplicate.

Table 2 Range of parameters for direct transesterification optimization.

Different parameters	Range
Moisture content (%)	0-50
Extraction temperature (°C)	50-90
Extraction time (min)	20-120
Solvent/ Algae biomass ml/g	3.5-10.5
Methanolic HCL (%)	2-12

EFFECT OF AMOUNT OF MOISTURE PRESENT IN ALGAE AND ITS EFFECT ON BIODIESEL YIELD

Algae was harvested using centrifugation method which was 19 wt% dry. In order to remove more moisture additional heating was done. The percentage of moisture content was calculated using equation 1 [16]. To check the effect of moisture different percentage of moisture was taken.

$$MP(\%) = \frac{P_i - P_f}{P_i} \times 100 \qquad (1)$$

RESULTS AND DISCUSSION

EFFECT OF DIFFERENT MOISTURE LEVELS ON FAME YIELD.

Wet algal mass 200mg (equivalent to dried algal mass) was directly transesterified using methanolic HCl and solvent. The maximum FAME yield (96%) was obtained when algal biomass has 0% moisture content and utilized for comparison with different percentage of moisture. Figure 1 shows the FAME yield with different moisture content for different methanolic HCl amounts. Methanolic HCl amount was varied from 1.5 ml to 4ml; maximum FAME yield was obtained at 3ml at all moisture level. As the moisture content increases in algal biomass there was significant decrease in biodiesel yield as shown in Figure 1. The result shows that moisture in algal biomass affects negatively on the biodiesel yield. However, increase in methanolic HCl results in a higher yield. Biodiesel yield is low at higher moisture content because as transesterification reaction is a reversible reaction presence of moisture content hydrolyzes the FAME in reverse direction producing methanol and free fatty acids. Moreover, presence of moisture in the biomass does not allow the oil to come in contact with solvents and thus inhibits the oil to become a part of the

reaction. Thus more amount of methanolic HCl results in a higher yield, as it enhances the oil contact with methanol. It was observed that at 4 ml methanolic HCl the FAME yield is same as at 3ml so using 4 ml solution is a wastage of money and uneconomical.

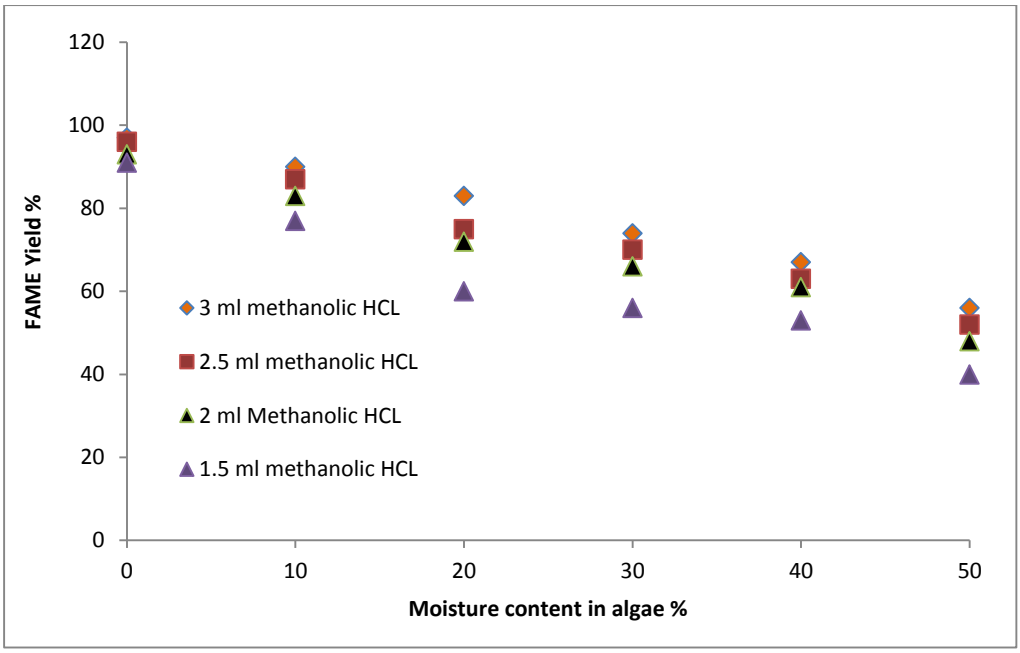
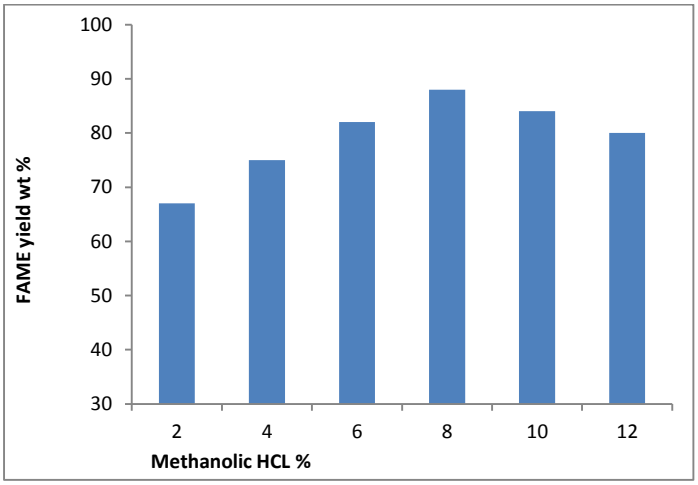


Figure 1 FAME yields of wet microalgae with different methanolic HCl amount at different moisture contents with fixed amount of solvent (7.5 ml).

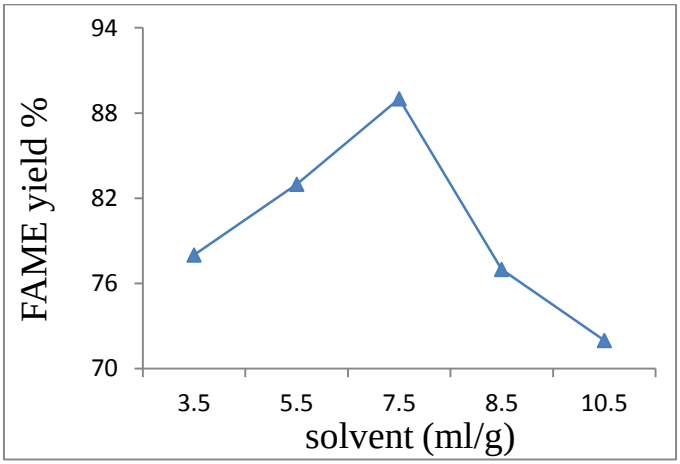
OPTIMIZATION OF DIFFERENT EXTRACTION PARAMETERS

For optimization purpose the moisture content in the algae was fixed at 20% and yield was analyzed with increase in the percentage of methanolic HCl and additional amount of solvent (methanol/ chloroform) affect was analyzed. The methanolic HCl percentage ranged from 2 to 12% and prepared according to Sukhija and Palmquist, (1988) [15]. The maximum FAME yield (88%) was obtained at 8% methanolic HCl as shown in Figure 2 (a). In in-situ transesterification, with methanolic HCl, it solves the purpose of both solvent as well as catalyst. HCl act as a catalyst while acetyl chloride act as a solvent so due to increase in the percentage of methanolic HCl, the effect of catalyst HCl enhances which results in higher yield. But beyond 8% methanolic HCl, that is at 10%, adverse effect was observed and yield was reduced because of the formation of undesired and unidentified derivatives. To determine the effect of solvent on the fatty acid methyl ester yield, the solvent amount was varied from 3.5ml to 10.5ml and optimum volume of solvent was 7.5ml/g of algae biomass as shown in Figure 2 (b). It is clear from the above data that FAME yield have direct relation with solvent. When the solvent is low the yield was low because presence of water in small volume of solvent makes the lipid and solvent contact harder, resulting in lower yield [17]. However, when the solvent volume was more than optimum value

it results in lower concentration of lipids in solvent and decrease in yield. To determine the effect of temperature on FAME yield in in-situ transesterification temperature was varied from 55^0C to 90^0C. The maximum yield was obtained at 85^0C to 90^0C with slight variation as shown in Figure 3, so 90^0C is chosen as the optimum temperature value where yield obtained was 86%. The yield was very low at 55^0C but as the temperature increases the yield also increases up to 90^0C. At 100^0C the FAME yield started decreasing because higher temperature breaks down the FAMEs. The results obtained above are in great accordance with other researcher work [4]. The reaction time greatly influences the FAME yield as shown in Figure 3. The yield was very low at 20 min which is 54%, with increase in time yield increases till 67% at 40 min, which further increases to 80% at 60 min. After 60 minutes yield was stable so optimum range of time 60-80 min was selected where maximum yield was obtained.



(a)



(b)

Figure 2 The FAMEs yield obtained by changing extraction parameters during one step transesterification of *Neochloris oleoabundans* microalgae species (a) the methanolic HCL range from 2 to 12% (b) the solvent volume from 3.5-10.5 ml/g;

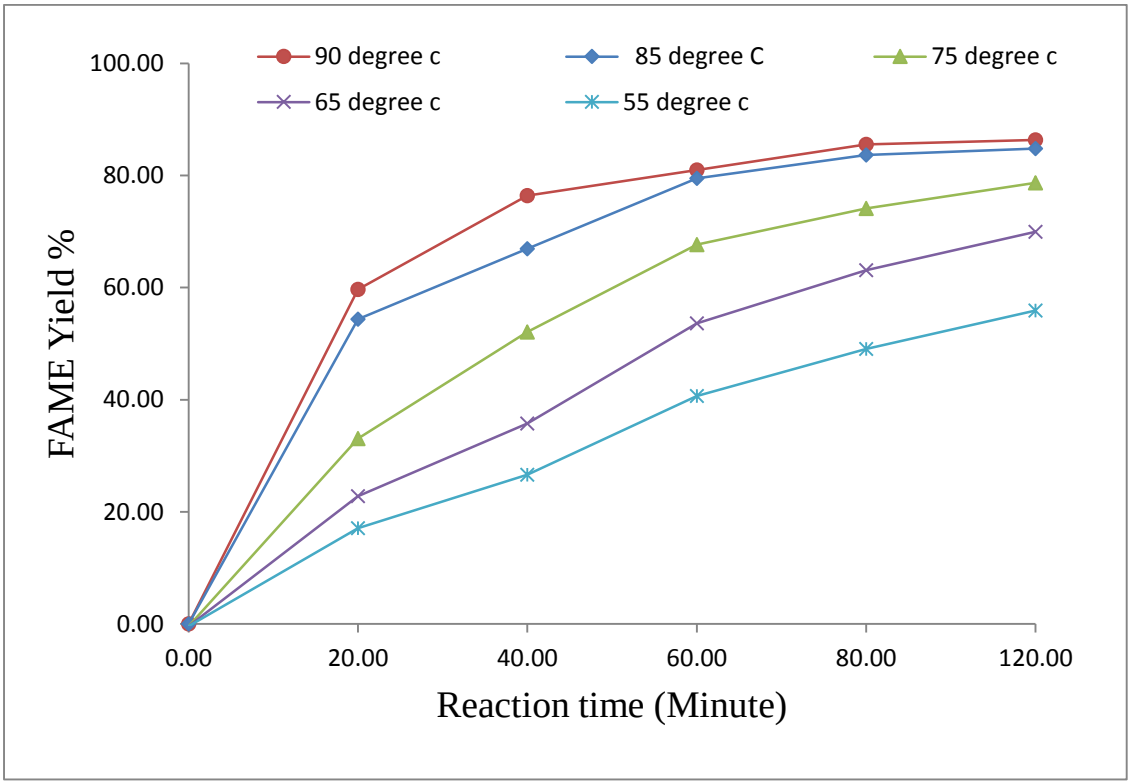


Figure 3 FAME yield obtained when range of reaction temperature varied from 55-100° C and the reaction time varied from 20-120min.

FAME AND BIODIESEL YIELD AT OPTIMAL CONDITION

All the extraction parameters at optimal condition (3ml methanolic HCl 8%, 7.5 ml solvent 90^0C temperature, and 80 min time) was used for direct transesterification of wet microalgal biomass having 20% moisture. The FAME yield and biodiesel yield obtained at optimal condition was high which found to be 90% and 84%.

ANALYSIS OF FAME PROFILES AND CONTENTS

GCMS was used to investigate the profile and contents of FAME. As illustrated in Table 3, the FAME profile of *Neochloris oleoabundans* analyzed by GCMS comprised of methyl myristate (14:0), Palmitic (16:0), methyl palmitoleate (16:1), Hexadecadienoic (16:2), Hexadecatrienoic (16:3), Stearic (18:0), methyl cis-9-octadecenoate (18:1), Linoleic (18:2), α-Linolenic (18:3). The above data demonstrate the major components of fatty acids which possess 88% of aggregate of fatty acids. This shows that *Neochloris oleoabundans* is good feedstock for production of biodiesel.

Table 3 Different biodiesel components produced during in-situ transesterification of wet *Neochloris oleoabundans* biomass at optimum reaction condition.

Fatty acid	FAME content %
C14:0	0.3
C16:0	30
C16:1	2.5
C16:2	1.5
C16:3	3.5
C18:0	3.1
C18:1	35
C18:2	23
C18:3	8

COMPARISON OF CONVENTIONAL AND MICROWAVE ASSISTED - TRANSESTERIFICATION METHOD

The work shows that with microwave assisted direct transesterification 90% yield could be achieved in less time. Time was reduced to 30 min which earlier took 80 min to complete the reaction. The biodiesel yield was increased by the use of microwave as compared to conventional direct transesterification method. This was due to disruption of hydrogen bonds caused by molecular dipole rotation in microwave. In microwave heating electromagnetic waves take part in reaction at molecular level resulting in enhancing the solvent diffusion, thus increase in lipid yield [18].

CONCLUSIONS

For direct transesterification of wet algae using methanolic HCl was examined to check the effectiveness of methanolic HCl when algal biomass contains high moisture. Result shows that higher FAME yield (60%) was obtained even when the moisture content in the algae was high that is 50%. As presence of water inhibits the reaction it was found that by addition of more amount of methanolic HCl slowly, FAME yield was enhanced by reducing the inhibition by moisture. However, addition of increased amount of methanolic HCl and solvent, the cost of the reaction increases, so optimization of extraction parameters was done. Finally investigation shows that at optimum condition in in-situ transesterification higher FAME yield was obtained reaching up to 84 % and reduces the cost. Thus one step transesterification is cost effective, fast and simple method to obtain the FAME yield.

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