

Methods

Identification of suitable strain:

A. Media for *Scandasmus dimorphus* algae growth

There were two types of media used in desired algal culture. Elemental compositions are following (Tables 1 and 2) [19].

B. Media preparation

Take above medium and make it (100 ml), Autoclave it at 121°C, 15 lbs pressure, for 15 min. Inoculate algal sample into four (100 ml) different conical flasks. Incubate it at normal RT, for 24 hours. Observe growth into different conical flasks. Select one algae containing flask which one has maximum growth and transfer it into 1000 ml media containing flask. Monitor growth of algae (day/day). After 10-15 days algal strain has to be used for further processing [19].

Algae harvesting:

A. Micro-screening

Algae with media in open pond take carefully on (250 μ , 500 μ) size sieves, filter and discard excess water, dry it in shade [19,20].

B. Centrifugation

Medium containing algae from open pond, if algal concentration is very low, take 30 ml centrifuge tube, transfer medium containing algae in to the tube. Centrifuge it with 4000 rpm, for 5 minutes, at room temperature. Discard supernatant and keep pellets for further oil processing [19,20].

Extraction of oil from algae:

A. Expeller press method

Take 500 g of shade dried algae. Transfer it into expeller machine for extraction of algal oil. Run the expeller machine. After some time collect algal oil from machine, make it for further transesterification process for biodiesel production [21].

B. Soxhlet extraction

Take 100 g dried powder of algae, keep it into soxhlet apparatus. Add 100 ml hexane solvent, to rupture cell wall of algae. Run the soxhlet (containing soxhlet-EMC / Method collected-EMC from round bottom flask). The oil has use for biodiesel production [19].

C. Ultrasonic-assisted extraction

Take 50 gm of dried algae and add 100 ml of ether in 250 ml beaker, provide ultra-sonic wave for 30 minutes, (ultrasonic waves are used to create cavitation bubbles in a solvent material) Ultra-sonic wave has also work as cell wall rupture of algae. Filter it with sieves, manually press algae to extract algal oil, algal oil has use to further transesterification process [21].

fume hood. You will see the lipids as yellow spots after about 5 min or so. Mark the edges of the spots with a pencil [22].

B. Paper chromatography

Take the Whatman No1 chromatography paper of appropriate size, Place it on a rough paper and with the help of pencil and scale draw a line leaving 1.5 cm from the bottom. Now on the line mark seven spots leaving 1.5 cm on either side of the edges. Now measure the distance between the spots carefully draw three small circles touching the

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Algal oil extraction

A. Ultrasonic-assisted extraction

Used ultrasonicator for algal oil extraction, upper layer is algal oil shown in the figure 6.

B. Soxhlet method for oil extraction

Used soxhlet apparatus for the extraction for oil from algae shown in figure 7.

C. Expeller press method for oil extraction

Used expeller machine for the extraction for oil from algae shown in figure 8.

Oil from different technique (Figure 9)

S.No.	Quantities of algae (gm)	Quantities of oil by different method (ml)		
		Soxhlet	Ultrasonicator	Expeller press
01	500	125	108	115
02	250	60	50	55
03	100	20	28	17



Figure 6: Ultrasonic assisted extraction.

Chromatographic technique

A. Thin layer chromatography (Figure 10)

RF factor=Distance traveled by solute (cm)/Distance traveled by the solvent (cm)

- A. RF factor of oil which was extracted by expeller method=10/10.7=0.93 cm
- B. RF factor of oil which was extracted by soxhlet method=9.2/10.7=0.85 cm
- C. RF factor of oil which was extracted by ultrasonicator method=5/10.7=0.46 cm
- D. RF factor of crude jatropha oil=9.7/10.7=0.90 cm
- E. RF factor of jatropha biodiesel=9.2/10.7=0.85 cm
- F. RF factor of karanja crude oil=9.7/10.7=0.90 cm
- G. RF factor of karanja biodiesel=10.2/10.7=0.95 cm

B. Paper chromatography technique (Figure 11)

RF factor=Distance traveled by solute (cm)/Distance traveled by the solvent (cm)
