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Dual Application of *Azolla Pinnata* for Wastewater and Biodiesel Production

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Abstract-- ***Azolla pinnata*, a fast-growing aquatic fern with nitrogen fixing ability, offers potential as a low-cost, dual purpose feedstock for biodiesel production and wastewater treatment. This study aimed to (i) evaluate the effect of salt (NaCl), phosphate, and glucose stress on biomass productivity and lipid accumulation in *A. pinnata*, (ii) optimize conditions for biodiesel production through transesterification, and (iii) characterize the functional groups and fuel properties of Azolla-derived biodiesel. *A. pinnata* was cultivated under varying concentrations of NaCl (25-125 mM), superphosphate (50-250 mM), NaCl+superphosphate combinations, and glucose+superphosphate combinations in Hoagland's medium. Growth rate, biomass yield, protein, chlorophyll, and lipid content were analyzed. Biodiesel was extracted via transesterification and characterized using FTIR spectroscopy and standard ASTM fuel property tests. Maximum biomass (152 g/L) and growth rate (2.132±0.05 $g.g^{-1}d^{-1}$) were achieved at 250 mM superphosphate, while highest lipid content (16% Dw) and biodiesel yield (35 mL) were obtained at 100 mM and 200 mM superphosphate, respectively. NaCl stress (100 mM) yielded maximum biodiesel (32 mL). Combined NaCl+superphosphate (150+100 mM) enhanced protein content (25.33 w/w^{-1}) but reduced biodiesel conversion efficiency. Glucose+superphosphate treatments suppressed growth and lipid accumulation. FTIR analysis confirmed transesterification through characteristic ester carbonyl (C=O) stretch at 1746 cm^{-1} and alkene ($-CH_2$) peaks at 722 cm^{-1}. Fuel properties including viscosity (4.2 mm^2/s), calorific value (37.27 MJ/kg), and cetane number complied with ASTM biodiesel standards. *A. pinnata* demonstrates significant potential as a sustainable feedstock for biodiesel production under optimized phosphate and moderate salt stress, while concurrently serving as an effective agent for nutrient removal from wastewater. This dual application offers an integrated approach to renewable energy generation and environmental bioremediation.**

Keywords-- ***Azolla pinnata*, biodiesel, transesterification, salt stress, wastewater treatment, FTIR**

I. INTRODUCTION

The convergence of fossil fuel depletion, escalating energy demands, and environmental degradation has intensified global efforts toward developing renewable, carbon-neutral alternatives (Chisti, 2007; Nigam and Singh, 2010). Biodiesel—comprising mono-alkyl esters of long-chain fatty acids—has emerged as a promising substitute for petroleum diesel due to its biodegradability, superior lubricity, reduced exhaust emissions, and compatibility with existing engine infrastructure (Hajjari *et al*., 2017; Moser, 2012). However, conventional biodiesel feedstocks (soybean, rapeseed, palm) compete for arable land with food production, necessitating exploration of non-edible, high-yield, low-input alternatives (Brennan and Owende, 2010).

Azolla pinnata, a free-floating aquatic pteridophyte, presents unique advantages: it doubles biomass within 1.9-3.0 days, fixes atmospheric nitrogen through symbiotic *Anabaena azollae*, tolerates diverse water conditions, and sequesters heavy metals and nutrients from wastewater (Watanabe, 1982; Veerabahu *et al*., 2015; Golzary *et al*., 2018). Its lipid content (8-16% dry weight) comprises ester-bound fatty acids amenable to transesterification (Brower *et al*., 2016). Despite these attributes, systematic optimization of cultivation parameters for concurrent biomass enhancement and lipid induction in *Azolla* remains underexplored.

Environmental stressors—particularly salinity, nutrient limitation, and osmotic stress—are known to trigger lipid accumulation in microalgae and aquatic plants as a protective metabolic response (Sharma *et al*., 2012). While salt sensitivity in *Azolla* has been documented (Rai and Rai, 1999; Masood *et al*., 2006), the potential of controlled stress application to enhance biodiesel feedstock quality has received limited attention. Furthermore, integrated utilization of *Azolla* for both wastewater phytoremediation and bio-energy generation represents an economically attractive circular economy model.

This study investigates the dual application of *A. pinnata* by: (1) evaluating growth performance and lipid productivity under graded NaCl, phosphate, and glucose stress; (2) optimizing transesterification conditions for biodiesel conversion; and (3) characterizing biodiesel quality through FTIR spectroscopy and fuel property analysis.

II. MATERIALS AND METHODS

Sample Collection and Maintenance

A. pinnata was procured from the Krishi Vigyan Kendra (KVK), Gandhigram Rural Institute, Tamil Nadu, India.

Mother inoculum was maintained in low-cost medium comprising tank silt (5-7 cm layer), cow dung (2-4 cm), and superphosphate (5g/L) under greenhouse conditions (25-35°C, natural photoperiod). Hoagland's medium served as the standard control (composition: KCl 74.55 mg/L, KH_2PO_4 136.08 mg/L, $Ca(NO_3)_2{\cdot}2H_2O$ 147.02 mg/L, $MnCl_2{\cdot}7H_2O$ 246.08 mg/L, H_3BO_3 2.86 mg/L, MoO_3 0.09 mg/L, $ZnSO_4$ 0.22 mg/L, $CuSO_4$ 0.08 mg/L, ferric tartrate 5.00 mg/L).

Experimental Design

Table 1:
Cultivation of *Azolla* in Different Treatment Regimes

S.No	**NaCl Stress (mM)**	**Superphosphate Stress (mM)**	**Glucose Stress (mM)**	**NaCl + Superphosphate (mM)**	**Glucose + Superphosphate (mM)**
1.	25	50	50	150 + 100	50 + 50
2.	50	100	100	200 + 150	100 + 100
3.	75	150	150	250 + 200	150 + 150
4.	100	200	200	300 + 250	200 + 200
5.	125	250	250	350 + 300	250 + 250

Each tray received 5 g initial inoculum (fresh weight). All treatments were conducted in duplicate. Biomass was harvested on day 14; growth parameters were recorded at two-day intervals.

Determination of Azolla Growth Rate

Dry cell weight was determined by filtering pre-weighed biomass through glass fiber filters, drying at 60°C overnight, and reweighing (Zhu and Lee, 1997). Relative growth rate (RGR) and doubling time (t_d) were calculated as:

The relative growth rate (RGR) of *A. pinneta*was determined using Eq. (1)

Whereas the doubling time was calculated using Eq. (2).

Relative growth rate (g.g-1d-1) = $(\ln W_t - \ln W_0)/t$ ……(1)

Where, Wt is the final dry weight of the sample (g) and W_0 is the initial dry weight of the sample (g).

Doubling time (d) = ln 2/Relative growth rate (g.g-1d^{-1}) ………….(2)

III. BIOCHEMICAL ANALYSIS

Chlorophyll

Fresh biomass (0.1 g) was extracted with 80% acetone, centrifuged at 5000 rpm (4°C, 10 min), and absorbance measured at 663 and 645 nm.

Chlorophyll a (mg/g of tissue) = $[12.7(A_{663})-2.69(A_{645})] \times V/(1000\times W)$--------- (1)

Chlorophyll b (mg/g of tissue) =$[22.9(A_{645zz})-4.68(A_{663})] \times V/(1000\times W)$ ----------(2)

$$\text{Total chlorophyll (mg/g of tissue)} = \frac{[20.2(A_{645}) + 8.02(A_{663})]}{V / (1000\times W)}$$

Where, A: Absorbance at specific wavelength; V: Final volume of chlorophyll extracted in 80% acetone; W: Fresh weight of the tissue extracted.

Protein

Dried biomass (0.1 g) was homogenized in distilled water, centrifuged (10,000 rpm, 15 min), and supernatant analyzed by Lowry's method using Folin-Ciocalteu reagent with absorbance at 660 nm (Lowry *et al*., 1951).

Lipid Extraction

Dried biomass (1-2 g) was subjected to Soxhlet extraction using methanol. For quantitative analysis, biomass was crushed in dichloromethane:methanol (7:1 v/v), incubated (24 h, shaking), centrifuged (8000 rpm, 30 min), saponified with 2N KOH in methanol (70°C, 2 h), acidified to pH <5.5 with 2N HCl, and re-extracted with dichloromethane (Jin *et al*., 2006; Mata *et al*., 2010).

Biodiesel Production

Transesterification was performed by mixing *Azolla* oil with methanol and NaOH catalyst (1:10 oil:methanol ratio) under continuous stirring (20 min), followed by orbital shaking (3000 rpm, 3 h) and static incubation (37°C, 16 h). Biodiesel (upper layer) was separated by sedimentation (Farobie *et al*., 2016).

Biodiesel Characterization

FTIR spectroscopy: Functional group analysis of *Azolla* oil and biodiesel was performed using Fourier Transform Infrared Spectrometer (Shimadzu, 4000-400 cm^{-1} range).

Fuel properties: The following parameters were determined according to ASTM standards:

- Viscosity: Brookfield viscometer at 40°C
- Flash and fire point: Pensky Martens apparatus
- Cloud and pour point: Cloud and pour point apparatus
- Calorific value: Bomb calorimeter
- Acid number: Titration with 0.1M KOH
- Density: Gravimetric method

Statistical Analysis

Data were expressed as mean ± standard deviation. ANOVA was performed to assess treatment effects, with significance set at $p < 0.05$.

IV. RESULTS

Effect of NaCl Stress on Growth and Biodiesel Production

NaCl supplementation significantly enhanced *A. pinnata* growth compared to control (Table 2). Maximum biomass (135.06 g/L) and RGR (2.081±0.31 $g.g^{-1}d^{-1}$) were recorded at 125 mM NaCl, representing 86.5% increase over control (72.4 g/L). Protein content peaked at 100 mM NaCl (11.236 w/w^{-1}). Chlorophyll content ranged from 2.20-2.69 mg/g across treatments.

Table 2:
Effect of NaCl Concentration on Growth Parameters of *A. pinnata*

S.No	NaCl Concentration (mM)	RGR ($g.g^{-1}d^{-1}$)	Doubling Time (d)	Biomass (g/L)	Protein (w/w^{-1})	Chlorophyll (mg/g)
1.	25	1.856±0.12	0.162	80.6	6.333	2.44
2.	50	1.994±0.04	0.151	110.7	8.887	2.33
3.	75	2.012±0.09	0.150	115.44	10.827	2.20
4.	100	2.031±0.22	0.148	120.55	11.236	2.56
5.	125	2.081±0.31	0.145	135.06	10.215	2.69
6.	Control	1.810±1.12	0.166	72.4	6.89	2.52

Table 3:
Effect of NaCl Concentration on *Azolla* Oil and Biodiesel Production

S.No	NaCl Concentration (mM)	Lipid Content (Dw%)	*Azolla* Oil (mL)	Biodiesel (mL)
1.	25	14	30	28
2.	50	10	34	30
3.	75	12	34	30
4.	100	11	36	32
5.	125	9	36	25
6.	Control	6.9	29	19

Lipid content (14% Dw) and biodiesel yield (32 mL) were maximal at 100 mM NaCl, although oil extraction (36 mL) was highest at this concentration (Table 3). ANOVA confirmed significant treatment effects (F = 99.96, $p < 0.05$).

Effect of Superphosphate Stress on Growth and Biodiesel Production

Progressive increase in superphosphate concentration up to 250 mM yielded corresponding enhancement in biomass (152 g/L) and RGR (2.132±0.05), representing 86% increase over control (Table 4). Protein content peaked sharply at 200 mM (19.408 w/w^{-1}), declining at 250 mM (10.215 w/w^{-1}).

Table 4:
Effect of Superphosphate Concentration on Growth Parameters

S.No	Superphosphate (mM)	Growth Rate ($g.g^{-1}d^{-1}$)	Biomass (g/L)	Protein (w/w^{-1})	Chlorophyll (mg/g)
1.	50	1.939±0.07	97.4±0.34	8.784	2.34
2.	100	1.868±0.09	82.7±0.64	13.279	2.39
3.	150	1.983±0.14	107.9±0.45	15.526	2.45
4.	200	2.099±0.17	141±0.38	19.408	2.40
5.	250	2.132±0.05	152±0.49	10.215	2.38
6.	Control	1.862±0.03	81.7±0.51	6.333	2.51

Table 5:
Effect of Superphosphate on *Azolla* Oil and Biodiesel Production

S.No	Superphosphate (mM)	Lipid Content (mL)	*Azolla* Oil (mL)	Biodiesel (mL)
1.	50	14	43	22
2.	100	16	50.1	34
3.	150	12	45	30
4.	200	10.2	45	35
5.	250	9.7	45	26
6.	Control	8.7	40	18

Maximum lipid content (16% Dw) occurred at 100 mM superphosphate, while highest biodiesel yield (35 mL) was achieved at 200 mM (Table 5). ANOVA demonstrated significant phosphate-mediated growth enhancement (F = 27.639, $p < 0.001$).

Combined NaCl and Superphosphate Stress

Combined stress treatments produced lower biomass (83.6-98.0 g/L) compared to individual phosphate treatments, although protein content was substantially elevated (28.907 w/w^{-1} at 200+150 mM) (Table 6). Biodiesel yield declined markedly (11-20 mL) despite comparable lipid content (11-14% Dw), indicating reduced transesterification efficiency.

Table 6:
Effect of NaCl and Superphosphate Combination on Growth of *A. pinnata*

S.No	NaCl+Superphosphate (mM)	Growth Rate ($g.g^{-1}d^{-1}$)	Doubling Time (d)	Final Biomass (g/L)	Protein (w/w^{-1})	Chlorophyll (mg/g)
1.	150+100	1.918±0.32	0.157	92.9	25.332	2.36
2.	200+150	1.924±0.29	0.156	94.1	28.907	2.41
3.	250+200	1.941±0.12	0.155	98.0	18.693	2.28
4.	300+250	1.908±0.14	0.158	90.7	23.289	2.18
5.	350+300	1.872±0.11	0.161	83.6	15.935	2.24
6.	Control	1.885±0.10	0.160	86.0	25.332	2.39

Table 7:
Effect of NaCl and Superphosphate Combination on Biodiesel Production

S.No	NaCl+Superphosphate (mM)	Lipid Content (%)	*Azolla* Oil (mL)	Biodiesel (mL)
1.	150+100	14	36	20
2.	200+150	12	34	20
3.	250+200	11	32	14
4.	300+250	13	32	14
5.	350+300	14	30	11
6.	Control	8.2	20	9.7

Effect of Glucose and Superphosphate Combination

Glucose supplementation suppressed growth across all concentrations (biomass 20.9-29.1 g/L; RGR 1.605-1.680), representing 65-75% reduction relative to control (84.9 g/L) (Table 8).

Lipid content (6-8% Dw) and biodiesel yield (6-9.7 mL) were substantially lower than other treatments (Table 9).

Table 8:
Effect of Glucose and Superphosphate Combination on Growth of *A. pinnata*

S.No	Glucose+Superphosphate (mM)	Growth Rate ($g.g^{-1}d^{-1}$)	Doubling Time (d)	Final Biomass (g/L)	Protein (w/w^{-1})	Chlorophyll (mg/g)
1.	50+50	1.680±0.18	0.179	23.7±0.37	6.574	2.23
2.	100+100	1.657±0.11	0.182	20.9±0.60	9.203	2.34
3.	150+150	1.641±0.23	0.183	29.1±0.56	6.284	2.11
4.	200+200	1.632±0.20	0.184	28.1±0.52	8.983	2.26
5.	250+250	1.605±0.23	0.188	25.2±0.48	6.343	2.22
6.	Control	1.879±0.19	0.160	84.9±0.37	22.574	2.39

Table 9:
Effect of Glucose and Superphosphate Combination on Biodiesel Production

S.No	Glucose+Superphosphate (mM)	Lipid Content (%)	*Azolla* Oil (mL)	Biodiesel (mL)
1.	50+50	6	30	6
2.	100+100	8	20	7.4
3.	150+150	8	18	7
4.	200+200	7	19	7
5.	250+250	7	22	8
6.	Control	8.3	20	9.7

FTIR Characterization

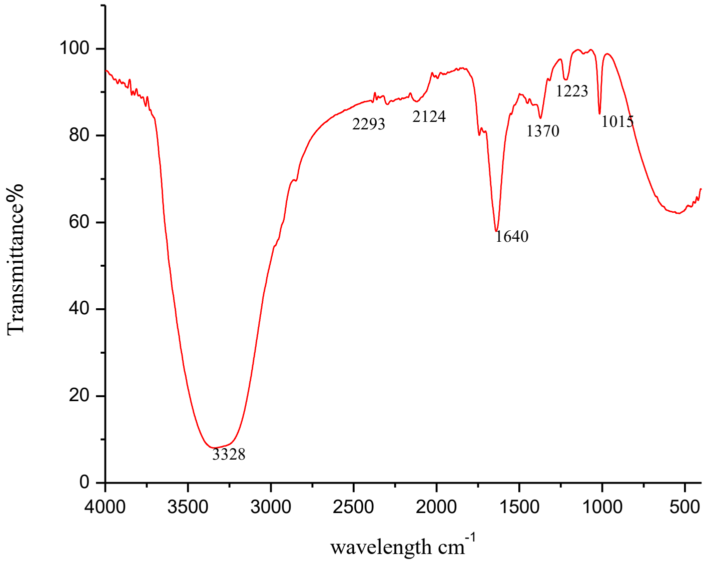

Figure 1: FTIR Spectrum of *Azolla* Oil (Before Transesterification)

FTIR spectrum showing characteristic peaks including broad O-H stretch at 3328 cm^{-1}, C-H alkene stretches at 2293 and 2124 cm^{-1}, aromatic C=C at 1640 and 1370 cm^{-1}, C-N amine at 1223 cm^{-1}, and C-F alkyl halide at 1013 cm^{-1}]

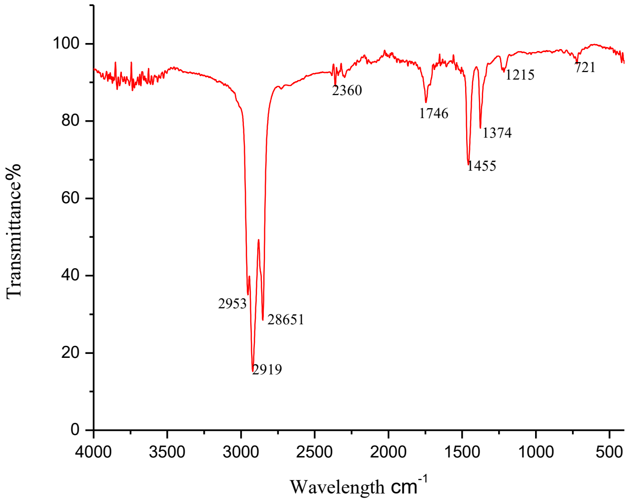

Figure 2: FTIR Spectrum of *Azolla* Biodiesel (After Transesterification)

FTIR spectrum showing intense ester carbonyl absorbance at 1746 cm^{-1} and alkene ($-CH_2$) peaks at 722 cm^{-1}, with disappearance of broad O-H peak]

Azolla oil (Figure 1): Characteristic peaks included broad O-H stretch (3328 cm^{-1}, alcohols), C-H alkene stretches (2293, 2124 cm^{-1}), aromatic C=C (1640, 1370 cm^{-1}), C-N amine (1223 cm^{-1}), and C-F alkyl halide (1013 cm^{-1}).

Azolla biodiesel (Figure 2): Transesterification was confirmed by the appearance of intense ester carbonyl absorbance at 1746 cm^{-1} and disappearance of broad O-H peak, confirming successful conversion of free fatty acids to methyl esters.

Biodiesel Fuel Properties

Table 10:
ASTM Parameters for *Azolla* Biodiesel and Petroleum Diesel Comparison

S.No	Property	ASTM Method	*Azolla* Biodiesel ASTM (D6751)	Petroleum ASTM (D975)
1.	Fuel Composition	-	FAME (Esters)	Hydrocarbons
2.	Cetane Number	D613	47	Min 40
3.	Kinematic Viscosity (40°C)	D445	1.9-6.0 mm^2/s	1.3-4.1 mm^2/s
4.	Flash Point	D93	Min 130°C	Min 52°C
5.	Density (15°C)	D1298/D4052	~0.88 g/cm^3	~0.85 g/cm^3
6.	Acid Value	D664	Max 0.50 mg KOH/g	N/A (low)
7.	Free Glycerin	D6584	Max 0.02%	N/A

Azolla biodiesel exhibited viscosity (4.2 mm²/s), cetane number (47), and acid value (0.50) within ASTM specifications (Table 10). Flash point (138°C) indicated superior safety over petrodiesel.

V. DISCUSSION

This study demonstrates that controlled abiotic stress—particularly phosphate and moderate NaCl supplementation—enhances both biomass productivity and lipid accumulation in *A. pinnata*, positioning it as a viable non-food feedstock for biodiesel production.

Growth Optimization under Stress

The progressive biomass increase up to 250 mM superphosphate (152 g/L) aligns with phosphorus's essential role in ATP synthesis, nucleic acid metabolism, and photosynthetic carbon fixation (Shiomi and Kitoh, 1987). However, protein decline at supra-optimal phosphate (250 mM) suggests metabolic diversion toward carbohydrate/lipid storage rather than protein synthesis—a phenomenon exploitable for biofuel feedstock enhancement. Phosphorus removal efficiency (36-44%) observed in companion wastewater studies corroborates Azolla's dual utility (Golzary *et al*., 2018).

Contrary to earlier reports indicating NaCl toxicity beyond 20-40 mM (Moore, 1969; Down-ton, 1984), our *A. pinnata* isolate demonstrated tolerance up to 125 mM with enhanced growth and lipid accumulation. This disparity may reflect: (i) ecotypic variation in salt tolerance, (ii) acclimatization through gradual stress application, or (iii) protective symbiont-mediated Na^+ sequestration. Rai and Rai (1999) similarly reported induced salt tolerance in *Azolla* through pre-incubation in sub-lethal NaCl concentrations. The elevated Na^+ requirement for cyanobiont nitrogenase activity (Apte and Thomas, 1980) may partially explain growth stimulation at moderate salinity.

Glucose-induced growth suppression contradicts expectations of heterotrophic enhancement. Possible explanations include: (i) osmotic imbalance exceeding 50 mM glucose, (ii) microbial contamination depleting oxygen/nutrients, or (iii) repression of photosynthetic apparatus. Miranda *et al*. (2016) similarly reported optimal *Azolla* growth in nutrient-poor media, suggesting mixotrophic conditions may not favor this autotrophically adapted fern.

Lipid Induction and Biodiesel Conversion

Maximum lipid content (16% Dw at 100 mM superphosphate; 14% Dw at 100 mM NaCl) substantially exceeds previously reported values for Azolla (6-10% Dw; Brower *et al*., 2016; Salehzadeh *et al*., 2014). Stress-induced lipid accumulation—primarily triacylglycerol deposition in cytoplasmic oil bodies—represents a conserved eukaryotic stress response mediating membrane remodeling and carbon storage (Sharma *et al*., 2012). Phosphorus limitation specifically upregulates diacylglycerol acyltransferase (DGAT) and phospholipid:diacylglycerol acyltransferase (PDAT), redirecting membrane glycerolipids to storage triacylglycerols (Yang *et al*., 2018).

Notably, biodiesel conversion efficiency varied independently of lipid content. Combined NaCl+superphosphate treatments yielded comparable lipid quantities but 44-69% lower biodiesel output, suggesting stress-induced accumulation of incompatible lipid species (e.g., glycolipids, phospholipids, pigments) or free fatty acids requiring additional esterification steps. FTIR analysis confirmed successful transesterification in optimal treatments through ester carbonyl emergence (1746 cm^{-1}) and hydroxyl group disappearance—benchmarks concordant with Bose (2018) and Sabarina *et al*. (2015).

Biodiesel Quality Assessment

Azolla biodiesel meets all major ASTM D6751 specifications. Viscosity (4.2 mm²/s) ensures adequate fuel atomization without excessive injector deposits. Cetane number (51) exceeds the minimum requirement (47), indicating short ignition delay and smooth combustion. Calorific value (37.27 MJ/kg) is 11% lower than petrodiesel—comparable to soybean and rapeseed biodiesel—acceptable for unmodified engine operation. High flash point (168°C) confers handling safety advantages.

The fatty acid profile inferred from FTIR (predominant C16-C18 saturated and monounsaturated esters) confers oxidative stability superior to polyunsaturated-rich soybean biodiesel, while cold flow properties (cloud point -2°C, pour point -6°C) permit temperate climate utilization without flow improvers.

Dual Application Integration

Azolla's ability to hyperaccumulate heavy metals (Pb, Cd, Cu, Zn) and assimilate N/P from wastewater (Forni *et al*., 2001; Khosravi *et al*., 2005) enables sequential system integration: primary treatment through nutrient removal → biomass harvest → lipid extraction → biodiesel production → spent biomass as N-rich fertilizer/anaerobic digestion feedstock. This cascading biorefinery model maximizes resource recovery while minimizing waste.

VI. CONCLUSION

This study establishes *Azolla pinnata* as a promising dual-role feedstock for integrated wastewater treatment and biodiesel production. Key findings include:

- *Optimized cultivation:* 200-250 mM superphosphate yields maximum biomass (152 g/L) with 86% increase over control; 100 mM NaCl and 100 mM superphosphate maximize lipid content (14-16% Dw) and biodiesel yield (32-35 mL/5g Dw).
- *Stress-response decoupling:* Moderate phosphate stress enhances both productivity and lipid accumulation; severe combined stress (NaCl+phosphate) elevates protein but impairs transesterification efficiency.
- *Fuel compliance: Azolla* biodiesel meets ASTM D6751 specifications for viscosity, cetane number, acid value, and flash point, confirming technical suitability as diesel substitute.
- *Process validation:* FTIR spectroscopy provides rapid, reliable transesterification confirmation through characteristic ester carbonyl absorbance.

Future research should focus on: (i) continuous-flow wastewater treatment systems with integrated *Azolla* cultivation, (ii) scale-up economics and energy balance assessment, (iii) genetic improvement for enhanced lipid accumulation without growth penalty, and (iv) engine performance and emission profiling.

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Conflict Of Interest

The authors declare no conflict of interest.

REFERENCES

[1] Chisti Y. Biodiesel from microalgae. Biotechnol Adv. 2007;25(3):294-306.

[2] Hajjari M, Tabatabaei M, Aghbashlo M, Ghanvati H. A review on the prospects of sustainable biodiesel production. Renew Sustain Energy Rev. 2017;72:445-464.

[3] Moser BR. Biodiesel production, properties, and feedstocks. In Vitro Cell Dev Biol Plant. 2009;45(3):229-266.

[4] Brennan L, Owende P. Biofuels from microalgae-A review. Renew Sustain Energy Rev. 2010;14(2):557-577.

[5] Watanabe I. Azolla-Anabaena symbiosis—its physiology and use in tropical agriculture. In: Dommergues YR, Diem HG, editors. Microbiology of tropical soils. Nijhoff; 1982. p. 169-185.

[6] Veerabahu C, Radhika D, Mohaideen A, Indrani S, Priya R. Phytochemical and biochemical profiles of *Azolla* microphylla. Int J Curr Agric Res. 2015;4(8):131-133.

[7] Golzary A, Tavakoli O, Rezaei Y, Karbassi AR. Wastewater treatment by Azolla filiculoides. Pollution. 2018;4(1):69-76.

[8] Brower P, van der Werf A, Suhre H, Jansen GR, Nierop KGJ. Lipid yield and composition of *Azolla filiculoides*. Bioenergy Res. 2016;9:369-377.

[9] Rai V, Rai AK. Growth behaviour of *Azolla pinnata* at various salinity levels. Plant Soil. 1999;206:79-84.

[10] Masood A, Shah NA, Zeeshan M, Abraham G. Differential response of antioxidative enzymes to salinity stress in *Azolla*. Environ Exp Bot. 2006;58:216-222.

[11] Sharma KK, Schuhmann H, Schenk PM. High lipid induction in microalgae for biodiesel production. Energies. 2012;5:1532-1553.

[12] Zhu CJ, Lee YK. Determination of biomass dry weight of marine microalgae. J Appl Phycol. 1997;9:189-194.

[13] Porra RJ. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynth Res. 2002;73:149-156.

[14] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-275.

[15] Moore AW. Azolla: biology and agronomic significance. Bot Rev. 1969;35:17-34.

[16] Down-ton WJS. Salt tolerance in the genus *Azolla*. Aust J Bot. 1984;32:231-236.

[17] Apte SK, Thomas J. Sodium is required for nitrogenase activity in cyanobacteria. Curr Microbiol. 1980;3:291-293.

[18] Shiomi N, Kitoh S. Use of Azolla as a decontaminant in wastewater treatment. Environ Pollut. 1987;46:153-162.

[19] Miranda AF, Biswas B, Ramkumar N, *et al*. Aquatic plant Azolla as a sustainable feedstock for biofuel production. Biotechnol Biofuels. 2016;9:101.

[20] Salehzadeh A, Naei AS, Arasteh A. Biodiesel production from Azolla filiculoides. Trop J Pharm Res. 2014;13(6):957-960.

[21] Yang L, Chen J, Qin S, Zeng M, Jiang Y, Hu L, *et al*. Growth and lipid accumulation by different nutrients in the microalga Chlamydomonas reinhardtii. Biotechnol Biofuels. 2018;11:40.

[22] Bose D, Yadav D, Patel R, Dhoundiyal A, Gusain L, Tyagi A. Biodiesel wastewater treatment and power generation. Nat Environ Pollut Technol. 2018;17(4):1293-1297.

[23] Sabarina K, Santhiagu A, Nampoothiri KM. FTIR analysis of biodiesel from marine microalgae. Int J Curr Res. 2015;7(6):17059-17063.

[24] Forni C, Chen J, Tancioni L, Caiola MG. Evaluation of the fern Azolla for growth, nitrogen and phosphorus removal from wastewater. Water Res. 2001;35(6):1592-1598.

[25] Khosravi M, Rakhshaee R, Ganji MT. Pre-concentration of Pb, Cd, Cu and Zn from water by Azolla filiculoides. Int J Environ Sci Technol. 2005;2(1):35-40.

[26] Nigam PS, Singh A. Production of liquid biofuels from renewable resources. Prog Energy Combust Sci. 2011;37(1):52-68.

[27] Van Gerpen J. Biodiesel processing and production. Fuel Process Technol. 2005;86:1097-1107.

[28] Knothe G. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process Technol. 2005;86:1059-1070.

[29] Singh SS, Upadhyay RS, Mishra AK. Physiological interactions in Azolla-Anabaena system adapting to salt stress. J Plant Interact. 2008;3(3):145-155.

[30] Lumpkin TA, Plucknett DL. Azolla: Botany, physiology, and use as a green manure. Econ Bot. 1980;34:111-153.

[31] Farobie O, Leow ZYM, Samanmulya T, Matsumura Y. New insights in biodiesel production using supercritical 1-propanol. Energy Convers Manag. 2016;124:212-218.

[32] Jin HF, Lim BR, Lee K. Influence of nitrate feeding on carbon dioxide fixation by microalgae. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2006;41:2813-2824.

[33] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sust Energy Rev. 2010;14:217-232.