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Waste Utilization and Biodiesel Production by the Green Microalga *Scenedesmus obliquus*[▽]

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Scenedesmus obliquus **was cultivated in three types of waste discharges to couple waste treatment with biodiesel production. The lipid pool accumulation was boosted to 1.0 g liter^{-1} against 0.1 g liter^{-1} for the control. The waste-grown *S. obliquus* showed an increase in the content of the saturated fatty acid pool, which is desirable for good-quality biodiesel.**

Biodiesel, which is produced from biomass by transesterification of triacylglycerols, is one of the most prominent renewable energy sources (14). Microalgae are emerging as one of the most promising resources of biodiesel, with a projected yield of 58,700 to 136,900 liter ha^{-1} year^{-1} (2). For microalga cultivation, the huge consumption of water resources and inorganic nutrients is costly (25). Addition of organic carbon, though found highly stimulatory for microalgal growth (4, 5, 10, 26), increases the feedstock cost (9). Thus, an economically acceptable and environmentally sustainable carbon source for alga-based biodiesel is currently needed.

One promising approach is to couple biodiesel production with wastewater treatment, as algae can be successfully cultivated in wastewaters (12). Cultivation of microalgae in swine wastes, dairy manure, and other animal residues has been reported by several authors (7, 15, 16, 18, 24); however, none

TABLE 1. Characteristics of MSST and FP discharges before and after inoculation of *S. obliquus*

Parameter	Waste discharge type	Days of incubation[a]					
		0	7	14	21	28	35
Orthophosphate (mg liter^{-1})	MSST	16.3	4.9	3.8	2.6	1.7	ND[b]
	FP	8.5	1.9	1.4	0.8	0.5	ND
Ammonium (mg liter^{-1})	MSST	131.4	15.1	9.8	7.2	3.1	ND
	FP	8.3	0.6	0.5	0.4	0.1	ND
Nitrate (mg liter^{-1})	MSST	85.2	30.5	4.0	ND	ND	ND
	FP	4.2	2.3	0.9	ND	ND	ND
Nitrite (mg liter^{-1})	MSST	0.5	ND	ND	ND	ND	ND
	FP	0.6	ND	ND	ND	ND	ND
Total organic carbon (mg liter^{-1})	MSST	11.8	7.2	6.1	5.5	3.5	2.3
	FP	17.2	13.5	11.4	8.3	3.8	2.7
BOD (O_2 mg liter^{-1})	MSST	86.7	40.1	29.6	22.8	17.4	13.5
	FP	118.7	61.4	49.2	40.2	21.1	14.5
COD (mg liter^{-1})	MSST	160.2	81.8	60.4	53.9	43.1	34.2
	FP	237.1	142.4	106.7	93.2	55.7	47.3
DO (mg liter^{-1})	MSST	1.2	2.7	3.6	4.4	5.1	5.9
	FP	3.2	4.1	5.8	6.1	6.5	6.8
pH	MSST	6.9	9.9	9.9	9.7	9.7	9.8
	FP	7.5	8.7	8.8	8.8	8.9	9.2

[a] Values represent averages of data from three independent determinations.
[b] ND, not detected.

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TABLE 2. Bioremediation efficiency of *S. obliquus* at different concentrations of PL on day 21 of incubation[a]

PL concn (g liter^{-1})	Orthophosphate (mg liter^{-1})	Ammonium (mg liter^{-1})	Nitrate (mg liter^{-1})	Nitrite (mg liter^{-1})	Total organic carbon (mg liter^{-1})
5	3.9 ± 0.2 (50.7 ± 0.8)	1.6 ± 0.2 (59.3 ± 0.9)	ND[b] (4.6 ± 0.3)	ND (0.7 ± 0.1)	31.2 ± 0.2 (58.6 ± 0.7)
10	4.1 ± 0.4 (104.2 ± 3.1)	1.7 ± 0.1 (120.9 ± 1.3)	ND (9.7 ± 0.2)	ND (1.5 ± 0.2)	68.5 ± 0.6 (116.4 ± 1.8)
15	4.8 ± 0.2 (151.4 ± 2.4)	2.2 ± 0.3 (182.1 ± 2.3)	ND (13.8 ± 0.6)	ND (2.3 ± 0.1)	87.2 ± 1.3 (175.2 ± 2.6)
20	5.1 ± 0.9 (206.4 ± 4.2)	2.5 ± 0.2 (237.4 ± 3.2)	ND (20.1 ± 0.5)	ND (2.9 ± 0.3)	111.2 ± 1.8 (230.7 ± 3.1)

[a] Values represent the averages ± standard errors of data based on three independent determinations. Values in parentheses represent data from determinations at day 0.
[b] ND, not detected.

of those studies represent experiments using poultry litter, except the work of Mahadevaswamy and Venkataraman (11), where bioconversion of poultry litter to biogas and utilization of the digester effluent for production of *Spirulina platensis* was investigated. The present article reports simultaneous biodiesel production and waste recycling with the green microalga *Scenedesmus obliquus* (Trup.) Kütz from the Gottingen culture collection (SAG 276-3a) by taking into account three types of waste: poultry litter (PL), fish pond (FP) discharges, and municipal secondary settling tank (MSST) discharges.

Characterization of waste discharges and nutrient removal efficiency of *S. obliquus*. The waste was characterized with respect to pH, dissolved oxygen (DO) content, biological oxygen demand (BOD), chemical oxygen demand (COD), and total organic carbon (TOC), PO_4^{3-}, NH_4^+, NO_3^-, and NO_2^- content following the standard protocols of American Public Health Association (1). Various quantities of PL were mixed with distilled water and with MSST and FP discharges (overnight) and were filtered through Whatman no. 44 filter paper. FP and MSST discharges alone (no PL added) and all the filtrates listed above were sterilized separately and used as growth media for *S. obliquus*. The nutrient removal efficiency of *S. obliquus* was analyzed in intervals of 7 days over a period of 35 days in a batch mode study using 50 ml of liquid medium (in a 150-ml Erlenmeyer flask) at 25 ± 2°C and cycles of 14 h of light (75 μmol photons m^{-2} s^{-1} photosynthetic active radiation) and 10 h of dark. *S. obliquus* grown in N 11 medium (21) under the conditions described above is referred to here as the control.

Nutrient concentrations in MSST and FP discharges decreased significantly over the experimental period, and on day 35, PO_4^{3-}, NH_4^+, NO_3^-, and NO_2^- reached levels that were under the detectable limits, corresponding to 100% biofiltration efficiency (Table 1). The pH and DO values showed rising trends as a consequence of carbon dioxide uptake and oxygen release during the process of algal photosynthesis (6). A fall in TOC, BOD, and COD values indicates that *S. obliquus*, in addition to its autotrophic mode, was able to utilize organic matters in a mixotrophic nutritional mode, as seen previously with *Chlorella sorokiniana* (8).

Nutrient removal by *S. obliquus* analyzed at different concentrations (5 to 20 g liter^{-1}) of PL supplementation showed the following trends on day 21 (Table 2): for NO_3^- and NO_2^-, 100% removal; for NH_4, 97 to 99% removal; for orthophosphate, 92 to 98% removal; and for TOC, 41 to 52% removal. As these experiments were performed under axenic conditions, the reduction in TOC is, therefore, attributed solely to utilization by *S. obliquus.*

Biomass and lipid yield of *S. obliquus* in wastes. The *S. obliquus* biomass was harvested by centrifugation (3,500 × *g* for 10 min) and dried under vacuum conditions before being analyzed for lipid content following the procedure described by Mandal and Mallick (13). A biomass yield of up to 2.3 g liter^{-1} was obtained in 20 g liter^{-1} PL-supplemented distilled water against 1.0 g liter^{-1} in the N 11 control (data not shown). This value was profoundly higher than the yield (0.3 to 0.5 g liter^{-1}) seen with typical microalgal cultivation (23). However, the maximum lipid yield (295 mg liter^{-1}) was reported in experiments using 10 g liter^{-1} PL-supplemented cultures. The addition of 5 g liter^{-1} PL to FP or MSST discharge resulted into a lipid yield comparable to that seen with 10 g liter^{-1} PL supplementation in distilled water (Table 3). Thus, these results imply that *S. obliquus* need not compete with other users of precious freshwater resources and that waste discharges such as sewage, fish pond discharge, and poultry litter could be used as suitable media for algal cultivation and lipid production.

TABLE 3. Biomass and lipid yield of *S. obliquus* in MSST and FP discharges with or without PL supplementation on day 21 of incubation[a]

PL concn (g liter^{-1}) or discharge category	Biomass (g liter^{-1})		Lipid yield (mg liter^{-1})[b]	
	MSST	FP	MSST	FP
N 11 (control)	1.1 ± 0.1a	1.0 ± 0.1b	122.1 ± 1.8a (11)	123.2 ± 2.0a (12)
Waste discharge	1.4 ± 0.2b	0.6 ± 0.1a	207.2 ± 2.5c (13)	110.1 ± 1.7a (19)
5	1.8 ± 0.2c	1.6 ± 0.2c	287.1 ± 2.6e (16)	279.3 ± 2.7e (17)
10	1.8 ± 0.2c	1.7 ± 0.2c	236.3 ± 2.1d (13)	228.6 ± 2.6d (14)
15	2.2 ± 0.2d	2.0 ± 0.2d	165.8 ± 1.8b (8)	189.1 ± 2.4c (10)
20	2.1 ± 0.2d	1.9 ± 0.2d	106.6 ± 2.3a (5)	137.1 ± 1.8b (7)

[a] Values represent averages ± standard errors of data based on three independent determinations. Values followed by the same letter did not differ significantly, as determined by Duncan's multiple-range test ($P < 0.05$). A separate analysis was done for each column.
[b] Values in parentheses represent lipid content percentages determined on the basis of percent dry cell weight.

TABLE 4. Biomass and lipid yield of *S. obliquus* in PL alone and in combination with MSST and FP discharges under optimized conditions[a]

Culture condition	Biomass (g liter^{-1})	Lipid yield (mg liter^{-1})[b]
N 11 control	1.1 ± 0.1a	118.7 ± 1.8a (11)
PL (20 g liter^{-1})	1.9 ± 0.2b	957.4 ± 2.2b (51)
MSST+PL (15 g liter^{-1})	2.0 ± 0.2b	1,048.9 ± 7.7c (53)
FP+PL (15 g liter^{-1})	1.8 ± 0.2b	947.4 ± 5.7b (54)

[a] Optimized conditions were as follows: when the stationary-phase culture was transferred to the medium containing 0.04 g liter^{-1} nitrate, 0.03 g liter^{-1} phosphate, and 1.0 g liter^{-1} thiosulfate for 8 days, the lipid content was boosted from 11% to 58% on the basis of comparisons to dry cell weight (13). Values represent averages ± standard errors based on three independent determinations. Values followed by the same letter did not differ significantly, as determined by Duncan's multiple-range test ($P < 0.05$). A separate analysis was done for each column.

[b] Values in parentheses represent lipid content determined on the basis of percent dry cell weight.

Lipid yield under optimized conditions. In our earlier report (13), transferring stationary-phase *S. obliquus* to a medium containing 0.04 g liter^{-1} nitrate, 0.03 g liter^{-1} phosphate, and 1.0 g liter^{-1} thiosulfate for 8 days was found to boost lipid accumulation. Thus, when *S. obliquus* was cultured in waste discharges up to the stationary phase and subsequently subjected to the optimized conditions at the second stage, the lipid pool accumulation was raised to 947 to 1,049 mg liter^{-1}, a range of values that is ~9-fold higher than that seen with the control (Table 4). The yield is comparable to that seen with *Nannochloropsis* sp. UTEX LB1999, where a lipid yield of up to 1,099 mg liter^{-1} was recorded in experiments using continuous nitrate-fed medium with 3% CO_2 purging (22).

Analysis of *S. obliquus* biodiesel. The acid-catalyzed transesterification of algal oil was carried out using a 60:1 molar ratio of methanol to oil, and the top organic layer was taken for analysis using a gas chromatograph (GC) (Clarus 500; Perkin-Elmer, Shelton, CT) equipped with an Elite-1 dimethylpolysiloxane capillary column (30 m by 0.25 mm by 0.25 μm) and a flame ionization detector used in split mode (1:50 [vol/vol]). The fatty acid methyl ester composition values of the biodiesel were determined by comparing the retention times of various standards (Sigma) and were confirmed by analysis using GC-mass spectrometry (GC-MS) (Autosystem XL; Perkin-Elmer, Shelton, CT). Methylpentadecanoate was used as the internal standard.

Palmitic acid was found as the major constituent in N 11-grown cultures (Table 5). Linolenic acid content was marginally higher than the specified limit of 12% (3). Interestingly, the waste-grown cultures showed a rise in palmitic acid content whereas linolenic and linoleic acid levels were reduced, indicating an increase in the oxidative stability of biodiesel. Piorreck et al. (17) and Pohl and Wagner (19) reported an increase in palmitic and oleic acid content using a low level of nitrogen. The level of nitrogen in waste discharges is significantly lower than that seen with N 11 medium. This might have been the cause for the rise in palmitic acid content of *S. obliquus* grown in waste discharges.

For a municipal wastewater treatment plant (Titagarh, Kolkata, India) that releases 183.6 m^3 wastewater h^{-1} (20), we calculated *S. obliquus* biodiesel production over a culture period of 21 days with an 8-day optimization period. Lipid pro-

TABLE 5. Fatty acid methyl esters of the biodiesel produced from *S. obliquus* grown under various conditions

Component	Fatty acid methyl ester content[a] (%)								
	Control	PL	MSST	FP	MSST+PL	FP+PL	PL[b]	MSST+PL[b]	FP+PL[b]
Palmitic acid	38.8	45.7	50.3	61.4	44.9	47.4	58.5	59.3	62.8
Oleic acid	35.4	33.9	32.4	30.4	34.2	32.5	28.7	29.7	27.0
Linoleic acid	10.8	9.8	7.2	1.7	9.5	9.2	4.6	3.1	2.7
Linolenic acid	15.0	10.6	10.1	6.5	11.4	10.9	8.2	7.9	7.5

[a] Values represent the average results from three independent determinations.

[b] Values represent growth under optimized conditions.

ductivity of 63,700 liter ha^{-1} year^{-1} could be projected with 62 algal ponds (150 by 20 by 0.5 m), assuming 11 cultivation cycles per year, leaving 45 days for cleaning and maintenance of the system. This could result in 45,600 liter ha^{-1} year^{-1} biodiesel yield (as biodiesel yield was 69% of crude lipid levels; unpublished data), with simultaneous treatment of 10×10^5 m^3 wastewater. Moreover, *S. obliquus* grown in waste discharges showed a rise in palmitic acid content, which is desirable for good-quality biodiesel.

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