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INVESTIGATION OF MICROALGAE CO-CULTURES FOR NUTRIENT RECOVERY AND ALGAL BIOMASS PRODUCTION FROM DAIRY MANURE

A. M. Asmare, B. A. Demessie, G. S. Murthy

ABSTRACT. *Treatment of waste streams using algae can minimize eutrophication by removing inorganic nutrients while producing biomass which can be used for biofuels, animal feed, and fertilizer production. While there are many studies that report the growth of individual algal strains in different media, there are relatively few studies that examine the performance of algae coculture. The objective of this research was to determine the growth parameters and nutrient sequestration profiles of* Chlorella vulgaris, Scenedesmus dimorphus*, and their coculture in wastewater from a dairy facility at two dilutions (10% and 25%).*

Average specific growth rates (and biomass concentrations) the S. dimorphus, C. vulgaris, *and their coculture were 0.263* d^{-1} *(0.290±0.059 g/L), 0.063* d^{-1} *(0.145±0.011 g/L), and 0.250* d^{-1} *(0.400±0.060 g/L)* d^{-1} *at 10% manure, and 0.232* d^{-1} *(0.543±0.149 g/L), 0.234* d^{-1} *(0.364±0.113 g/L), and 0.289* d^{-1}*(0.612±0.255 g/L) at 25% manure, respectively. Based on the results it was evident that the strains* S. dimorphus *and* C. vulgaris *have different capacities for accumulation of biomass production (*S. dimorphus *is higher), lipid accumulation (*S. dimorphus *is higher), chlorophyll (*C. vulgaris *is higher), total suspended solids (TSS) (*C. vulgaris *is higher), and volatile suspended solids (VSS) (*S. dimorphus *is higher). It was found that mixed coculture had higher biomass growth, specific growth rate, and removal efficiency of nitrogen, phosphorous, and TSS for the 25% dairy wastewater. The results were similar for 10% dairy wastewater except for the specific growth rate and nitrogen removal efficiency which were higher for the* S. dimorphus *monoculture. These capacities can be leveraged in mixed coculture to achieve higher treatment efficiencies compared to monocultures. The results can inform managers of agricultural and municipal wastewater facilities as they make decisions about whether to include algal technology in future upgrades and expansion.*

Keywords. *Wastewater,* Chlorella vulgaris, Scenedesmus dimorphus*, Microalgae coculture, Nitrate removal, Phosphate removal.*

Wastewater treatment systems exist in most developed nations but are energy and capital intensive. For example, to treat 0.1-1 MGD (million gallons per day) of wastewater using conventional wastewater treatment system (mechanical systems) requires use of 80-240 KWh/MGD of electricity and operating costs can range from \$0.70-\$2.9/MGD (Muga and Mihelcic, 2008). Many installations especially in the developing countries are abandoned due to the high operation and maintenance costs (Kadlec and Knight, 1996). Designing wastewater systems that incorporate appropriate technology and economic constraints would be the key to successful operation of wastewater treatment plants in many developing countries such as Ethiopia. In this context, previous research demonstrated that large-scale algae cultivation solely for energy production was uneconomical and suggested future research into waste-stream integration (Rose, 1999). Production of biofuels from algae using wastewater could provide a clean, sustainable and low cost solution for producing bioenergy/biofertilizers while simultaneously treating wastewater. Such systems will also increase the sustainability through recovery and recycle of nutrients such as nitrogen and phosphorous from wastewater.

Researchers have investigated the use of algae in open raceway ponds using municipal wastewater, agricultural wastewater, and dairy and industry effluents (Kebede-Westhead et al., 2004; Mulbry et al., 2008). Open raceway ponds although preferred for low overall costs have various limitations such as contamination by other microalgae species, zooplankton, lower stability of cultures, and productivity compared to closed photobioreactors. While monocultures of algae have been extensively investigated, in natural ecosystems the algae coexist in mixed coculture and not as monocultures (Kumar and Goyal, 2008; Hiroyuki et al., 2010). In addition, it was observed that different species of algae have different nutrient sequestration profiles and therefore could be complemen-

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tary in terms of nutrient utilization and result in a more robust growth and culture (Kebede-Westhead et al., 2003; Woertz et al., 2009).

While there are many studies that report the growth of individual strains in different media (Gonzalez et al., 1997; Pizarro et al., 2002; Mulbry et al., 2005; Kebede-Westhead et al., 2006; Levine et al., 2011; Pittman et al. ,2011), there are few studies (Chinnasamy et al., 2010; Gonzalez et al., 2008; Godos et al., 2010) that characterize the performance of algae cocultures and consortia. The objective of this research was to determine the growth parameters of *Chlorella vulgaris*, *Scenedesmus dimorphus* and their cocultures in wastewater from a dairy facility at two dilutions (10% and 25%).

These two algal strains (table 1) were selected as they are commonly found in many the wastewater treatment facilities. These strains were chosen due to their demonstrated robust growth characteristics in waste streams and potential used for biofuel, animal feed, and fertilizer applications. This study would provide results on the differences in the growth, cell density, nutrient absorption, lipid, and chlorophyll content of the two strains of algae in monocultures and coculture scenarios under various growth conditions.

Table 1. Chemical composition of algae expressed on a dry matter basis (%).[a]

Strain	Protein	Carbohydrate	Lipid Content	Nucleic Acid
Scenedesmus dimorphus	8-18	21-52	16-40	-
Chlorella vulgaris	51-58	12-17	14-22	4-5

[a] Source: Becker, 2007.

MATERIALS AND METHODS

OVERVIEW OF EXPERIMENTS

The experimental setup consisted of 1L cylindrical glass photobioreactors with 800 mL working volume (fig. 1). There were nine photobioreactors with regulation of light, temperature, carbon dioxide, and pH. A constant source of light at 200-380 µE $m^{-2}s^{-1}$ intensity was provided using fluorescent lamps and eight LEDs (four red and four blue LEDs at wavelength of 660 and 430 nm, respectively) as light sources. Air (95%) and CO_2 (5%) were supplied from the bottom of the photobioreactor to prevent algae from settling. The entire setup was placed in a temperature regulated room with a constant temperature of 25°C and had no external light other than the sources described above.

A photoperiod of 12 h (12 h light:12 h dark in 24 h period) was used in all experiments. Inoculum was added at 10% media volume level in all treatments. The volume of the photobioreactors was checked daily and make up water was added to each photobioreactor to replace the water that had been removed due to sampling and evaporation. Two sets of experiments with 10% and 25% dairy wastewater as the sole nutrient source were conducted to determine the growth parameters for individual algae strains and mixed cocultures.

Algae Strains

Two microalgae strains used in this study *Scenedesmus dimorphus* (UTEX #1237) and *Chlorella vulgaris* (UTEX #2714) were obtained from UTEX culture collection. The algae strains were maintained in Proteose medium specified in the UTEX protocol. Briefly, the media was prepared by mixing 850 mL of distil water, $NaNO_3$ (2.94mM), $CaCl_2 \cdot 2H_2O$ (0.17mM), $MgSO_4 \cdot 7H_2O$ (0.3mM), K_2HPO_4 (0.43mM), KH_2PO_4 (1.29mM), NaCl (0.43mM), and Proteose Peptone (1 g/L). The proteose medium was autoclaved prior to use to prepare media for maintaining the cultures. Only diluted dairy wastewater was used as a substrate to grow algae strains in the subsequent experiments as described below.

The microalgae cultures were inoculated at 10% (v/v) of the photobioreactor working volume. Initial inoculum cell concentrations were ~7.5×10^6 and 4.2×10^6 cells/mL for *S. dimorphus* and *C. vulgaris,* respectively. The algae coculture inoculum was prepared using a 50% volume mixture of the two individual strain inoculums and had an initial cell concentration of 6.3×10^6 cells/mL.

Substrates

Free-stall barn flush water dairy manure effluent was collected from a Dairy farm located at Oregon State University, USA. Effluent was homogenized by mechanical agitation, filtered (Whatman 1454-150 filter with 22 µm pore size) and subsequently was stored at 4°C for further use. The dairy manure filtrate had total suspended solid

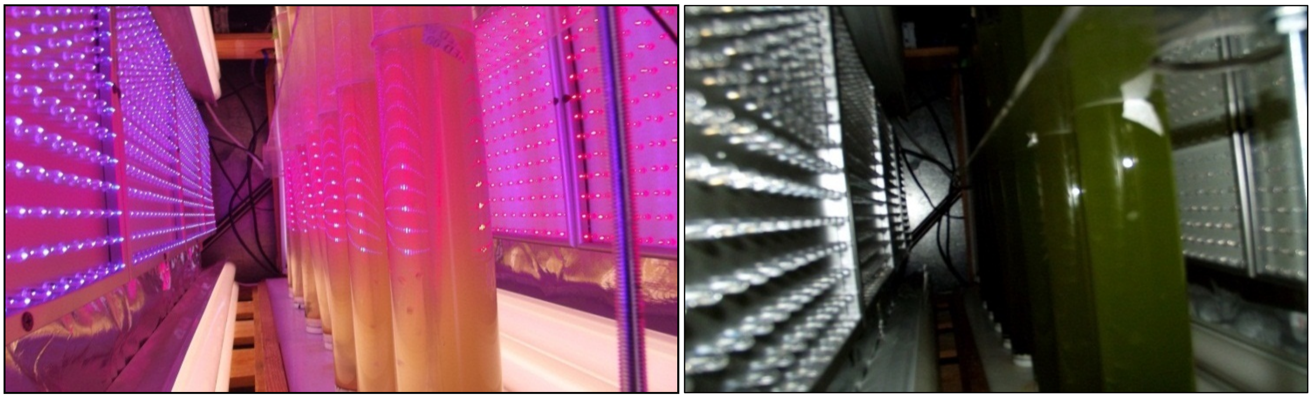

Figure 1. Photobioreactors used in the experiments (with lights on and off).

(TSS) 3177.78±591.15 mg/L, volatile suspended solid (VSS) 3020±605.94 mg/L, soluble-orthophosphate (PO_4^{-3}-P) 67.18±5.04 mg/L, nitrate (NO^-_3-N) 30.96±3.04 mg/L. Dairy manure effluent filtrate was used in all experiments as the sole nutrient source.

EXPERIMENTAL MEASUREMENTS

Periodic samples were drawn to measure TSS, VSS, PO_4^{-3}-P, NO^-_3-N, algal cell, biomass, chlorophyll and crude lipid concentrations. The temperature and pH were monitored daily *in-situ*. Spectrophotometric absorbance readings were taken daily using a 5 mL sample. Cell counts were measured on 0, 2, 3, 4 and 6 days. Measurements for chlorophyll were taken on days 0, 2, 4 and 6 using a 10mL sample. Measurements for TSS, VSS, PO_4^{-3}-P, and NO^-_3-N, were taken on days 0, 3, and 6 days using a 30 mL sample. Crude lipid measurements were performed at the conclusion of the experiments. The protocols to conduct these measurements are briefly described below.

Algae Cell and Cell Mass Concentration

Numbers of cells per unit volume was determined using cell heamocytometer (APHA, 2005). The optical density of the sample was measured at 625 nm using a spectrophotometer (Spectronic GENESYS 10Bio) and optical path length of 0.01 m. Algae biomass concentration was estimated using the following eq. (eq. 1):

$$B = 0.38 OD_{625} \tag{1}$$

where

OD_{625} is the optical density at 625 nm.

B is the algae biomass concentrations (g/L).

Rate of specific growth rate and cell doubling time were estimated as follows (eqs. 2 and 3).

$$\mu = log\left(\frac{B_t}{B_0}\right) / (T_t - T_0) \tag{2}$$

$$T_d = \ln(2)(T_t - T_0) / \ln\left(\frac{N_t}{N_0}\right) \tag{3}$$

where

B_0 and B_t = initial and final algae biomass concentrations (g/L), respectively.

N_0 and N_t = initial and final cell concentrations (cells/mL), respectively.

T_0 and T_t = initial and final times (day), respectively.

μ and T_d = specific biomass growth rate (day^{-1}) and cell doubling time (day^{-1}), respectively.

Chlorophyll Content

Algal cells were extracted in ethanol and chlorophyll content was determined spectrophotometrically by measuring the absorbance of ethanol extract against an ethanol blank at 649 and 665 nm using a spectrophotometer (Spectronic GENESYS 10Bio, Thermo Scientific, West Palm Beach, Fla.). The chlorophyll *a, b,* and total chlorophyll concentrations (g/L) were determined using the following equations (Minocha et al., 2009):

$$Chlorophyll\ a = 13.36\ A_{665} - 5.19 A_{649}$$

$$Chlorophyll\ b = 27.43\ A_{649} - 8.12 A_{665}$$

$$Total\ Chlorophyll = Chlorophyll\ a + Chlorophyll\ b \tag{4}$$

where

A_{649} = the absorbance for chlorophyll at 649,

A_{665} = the absorbance for chlorophyll at 665.

Crude Lipid Content of Algae

Crude lipid content of the algae was measured during various growth stages to determine lipid productivity. Extraction of crude lipids was performed using ANKOM lipid extraction system (Model XT15, ANKOM, Macedon, N.Y.). Estimation of the total lipid content was made as fraction of total dry cell biomass using a gravimetric method.

$$Crude\ Fat(\%) = \frac{\Delta W}{W} 100 \tag{5}$$

where

W = original weight of sample,

ΔW = weight loss after extraction with petroleum ether and drying.

Water Quality Analyses

All water quality analyses were conducted on cell free samples obtained by filtering original samples through a Whatman 1454-150 filter with 22 µm pore size. The TSS and VSS concentrations were determined gravimetrically as per the Standard Method 2540 D (APHA 2005) immediately after sampling. Orthophosphate (PO_4^{-3}-P), and nitrate (NO_3^--N) were measured based on the ascorbic acid and cadmium reduction methods (method 4500-P-D) (APHA, 2005) using Hach PhosVer™ reagent and NitraVer™ reagent, respectively (Hach Company, Loveland, Colo.). Removal rates for those parameters were calculated using:

$$Removal\ Effeciency(\%) = \frac{Intitial\ Concentration - Final\ Concentration}{Intitial\ Concentration} \times 100 \tag{6}$$

Statistical Analysis

All experiments were carried performed in triplicate, and all data represent the mean and statistical significance of three replicate measurements except where noted. Final day data were normalized by dividing the concentrations (mg/L) by final biomass concentrations (g/L) to obtain biomass normalized data for a consistent comparison among treatments with different final biomass concentrations.

RESULTS AND DISCUSSION

Raw unsterilized dairy wastewater was used as the sole nutrition media for the algae and the experimental conditions were relatively steady (21°C to 22.5°C, 7.1-8.2 pH, light intensity of 200-380 µE $m^{-2}s^{-1}$ as measured on the surface of the cultures) during all the experiments. The temperature and pH of the cultures was within the optimum growth range suggested for most strains of the algae (Soeder, 1981; Grobbelaar, 1982; Fontes et al., 1987; Borowitzka, 1998; Chevalier et al., 2000).

The microalgae growth for each treatment was observed throughout the treatment period of seven days (figs. 2 and 3). For the media with 10% manure as the sole nutrient source the final (initial) algal cell densities were 11.87±0.68 (7.52±0.58), 5.10±0.44 (4.20±0.50), and 14.87±0.73 (6.35±1.32) million cells/mL for *S. dimorphus, C. vulgaris*, and the coculture mix, respectively, at the end of the 7 day growth period. Similarly it was observed that for media with 25% manure, the comparative cell densities were 10.07±0.26 (7.05±0.10), 4.18±0.25 (3.68±0.08), and 9.78±0.25 (5.33±0.10) million cells/mL for *S. dimorphus, C. vulgaris*, and the coculture mix, respectively, at the end of the 7 day growth period. In the two treatments, the *S. dimorphus* strain had higher cell densities compared to *C. vulgaris.* The maximum mean cell counts occurred at the 10% manure concentration for all three strain treatments compared to 25% manure. However it was observed that the cell densities of the coculture exceed the average cell densities of the individual cultures at all sampling times indicating a synergistic interaction between the strains (table 2).

Biomass concentrations at the end of 7 day growth period for the media with 10% manure as the sole nutrient source were 0.290±0.059, 0.145±0.011, and 0.400± 0.060 g/L for *S. dimorphus, C. vulgaris*, and the coculture mix, respectively. Similarly it was observed that for media with 25% manure, the comparative biomass concentrations were 0.543±0.149, 0.364±0.113, and 0.612±0.255 g/L for *S. dimorphus, C. vulgaris*, and the coculture mix, respectively, at the end of the 7 day growth period. Average specific growth rates the *S. dimorphus, C. vulgaris*, and coculture mix were 0.263, 0.063, and 0.250 d^{-1} at 10% manure, and 0.232, 0.234 and 0.289 d^{-1} at 25% manure, respectively (table 3). These growth rates were lower compared to the growth rates in the Bristol medium (0.3644 d^{-1}) under axenic conditions (Lau et al. 1997). In all cases, average biomass growth rates were significantly higher while doubling times were correspondingly lower for the coculture mix compared to both the strains, further strengthening the hypothesis that the coculture have a better growth profile compared to strains individually.

Chlorophyll content of cells is an important determinant of their overall autotrophic growth efficacy. Chlorophyll a concentrations at the end of the 7 day growth period for the media with 10% manure as the sole nutrient were 1.766±0.538, 0.965±0.062 and 2.824±0.778 g/L for *S. dimorphus, C. vulgaris,* and the coculture mix, respectively (table 4). Chlorophyll b and total chlorophyll concentrations were significantly higher for media with 25% manure for all treatments. Interestingly, biomass normalized chlorophyll concentrations higher for *C. vulgaris* compared to *S. dimorphus* and coculture mix at both concentrations of manure.

The crude lipid content was significantly higher in *S. dimorphus* (23.5% and 22.2%) and mixed coculture (25.4% and 25.5%) compared to *C. vulgaris* (7.9% and 9.9%) cultures at 10% and 25% manure concentrations, respectively (table 2). Similarly lipid productivity of *S. dimorphus* (68.5 and 138.5 mg/L,) was higher compared to

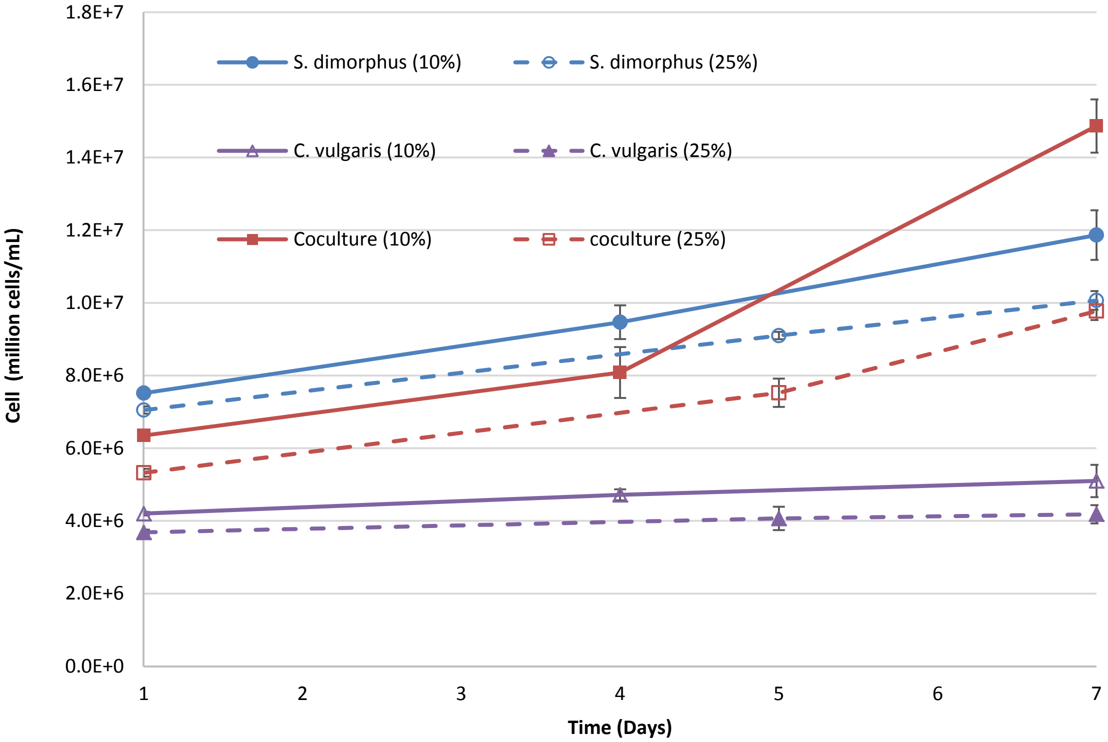

Figure 2. Algae cell concentrations (cells/mL) during growth in dairy manure (10% and 25% concentration).

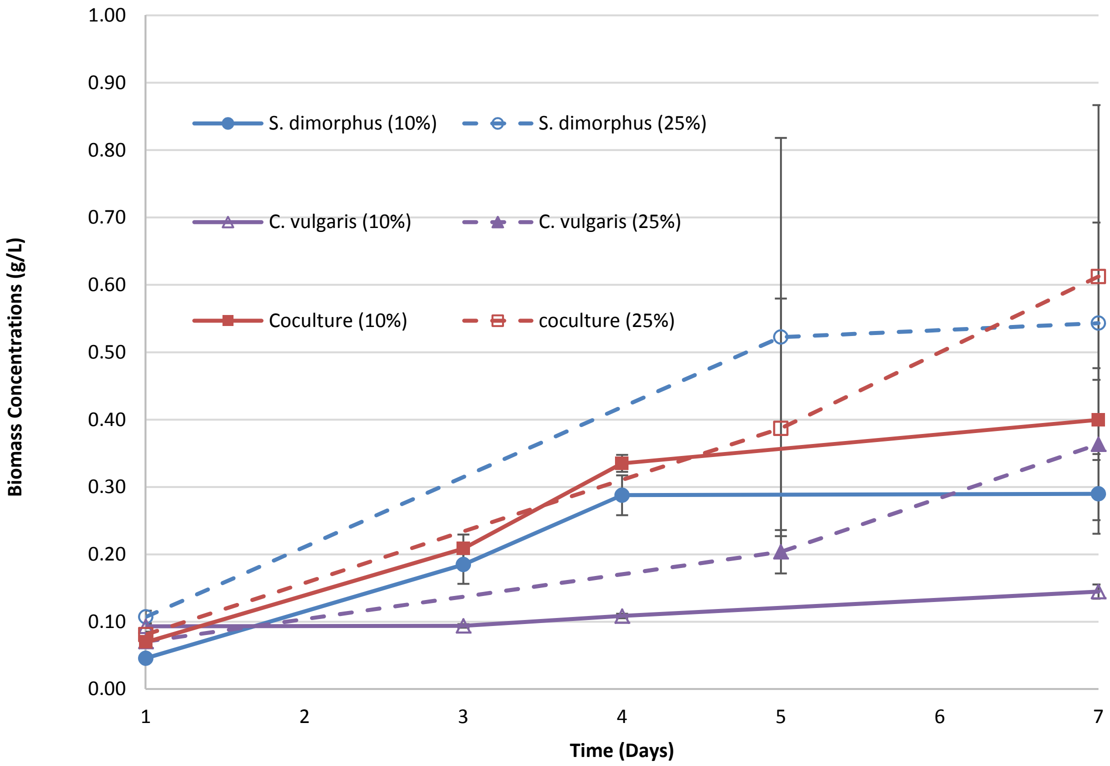

Figure 3. Algae biomass concentrations (g/L) during growth in dairy manure (10% and 25% concentration).

Table 2. Algae cell count (cells/mL), biomass (g/L) and crude lipid content (%).

		Cells Concentration (million cells/L)		Biomass Concentration (g/L)		Lipid Content (%)
Treatment	Algae	Initial	Final	Initial	Final	Final
10% Dairy Wastewater	*S. dimorphus*	7.52±0.06	11.87±0.68	0.046±0.0052	0.290±0.0591	23.5
	C. vulgaris	4.20±0.05	5.10±0.44	0.093±0.0027	0.145±0.0106	7.9
	Mix	6.35±0.13	14.87±0.73	0.070±0.0052	0.400±0.0595	25.4
25% dairy wastewater	*S. dimorphus*	7.05±0.10	10.07±0.25	0.107±0.0093	0.543±0.1494	22.2
	C. vulgaris	3.68±0.76	4.18±0.25	0.071±0.0075	0.364±0.1128	9.9
	Mix	5.33±0.11	9.78±0.25	0.081±0.0043	0.612±0.2545	25.5

C. vulgaris (11.46 and 36.04 mg/L) at 10% and 25% manure concentrations, respectively. These results indicate that if the purpose of producing algae biomass is solely to produce lipids from algal biomass, *S. dimorphus* may be a better alternative compared to monoculture of *C. vulgaris*. However, in the waste treatment scenario investigated in this article, the primary aim is removal of nitrogen and phosphorous and a reduction of TSS and VSS.

Removal efficiencies of nitrate (NO_3^--N) from the dairy wastewater at 10% and 25% manure concentration were 83.2%, 4.1%, 65.7%, and 67.7%, 55.3%, 70.7% for *S. dimorphus, C. vulgaris,* and coculture mixture, respectively, after 7 days of growth (table 5). Similarly phosphate removal efficiency measured as total orthophosphate equivalents (PO_4^{-3}-P) were 82.9%, 20.8%, 84.6% (at 10% manure concentration), and 22.0%, 12.0%, and 56.8% (at 25% manure concentration) for *S. dimorphus, C. vulgaris,* and coculture mixture, respectively, after 7 days of growth.

Table 3. Average cell division, specific growth rate and doubling time of algae.

Treatment	Algae	Cell Division Rate (k)	Specific Growth Rate (µ)	Doubling Time (day)
10% dairy wastewater	*S. dimorphus*	0.106	0.263	10.63
	C. vulgaris	0.045	0.063	24.99
	Mix	0.198	0.250	5.70
25% dairy wastewater	*S. dimorphus*	0.083	0.232	13.62
	C. vulgaris	0.030	0.234	38.12
	Mix	0.142	0.289	7.99

The initial orthophosphate phosphorus concentration (6.88 mg/L) of *S. dimorphus*, *C. vulgaris,* and their cocultures for 10% manure were reduced to 1.17, 5.45, and 1.06 mg/L, respectively, in 7 days. At 25% manure concentration the initial phosphate concentration (16.39 mg/L) reduced to 12.78, 14.42, and 7.087 mg/L, respectively, in 7 days for *S. dimorphus*, *C. vulgaris*, and their cocultures. Initial total nitrate concentrations of 3.12 mg/L at 10% manure treatment were reduced to 0.52, 2.99, and 1.07mg/L, for *S. dimorphus*, *C. vulgaris*, and their cocultures, respectively. For 25% manure treatment, the initial total nitrate concentrations of 7.69 mg/L were reduced to 2.48, 3.43, and 2.25 mg/L for *S. dimorphus*, *C. vulgaris*, and coculture mix, respectively, in 7 days (table 5). The final values of total nitrogen and orthophosphate in the media after algal growth are lower than the

Table 4. Chlorophyll a, b total chlorophyll (mg/L) and biomass normalized total chlorophyll (mg /g biomass) concentrations.

		Chlorophyll a Concentration (mg/L)		Chlorophyll b Concentration (mg/L)		Total Chlorophyll Concentration (mg/L)		Biomass Normalized Total Chlorophyll (mg/ g biomass)	
Treatment	Algae	Initial	Final	Initial	Final	Initial	Final	Initial	Final
10% dairy wastewater	*S. dimorphus*	0.363±0.183	1.766±0.538	0.435±0.290	0.788 ± 0.29	0.798	2.554	17.400	8.812
	C. vulgaris	0.107±0.056	0.965±0.062	0.526±0.036	1.02 ± 0.036	0.633	1.985	6.785	13.710
	Mix	0.161±0.312	2.824±0.778	0.480±0.165	0.904 ± 0.165	0.641	3.727	9.208	9.327
25% dairy wastewater	*S. dimorphus*	0.656±0.280	9.473±4.872	0.281±0.166	3.654 ± 0.166	0.937	13.126	8.745	24.167
	C. vulgaris	0.649±0.833	8.555±2.806	0.172±0.056	4.651 ± 9.174	0.821	13.206	11.617	36.327
	Mix	0.431±0.00	9.188±1.239	0.224±0.00	3.872 ± 0.00	0.654	13.059	8.085	21.325

Table 5. Nitrate and Phosphate removal efficiencies.

		Nitrate			Phosphate		
		Concentration (mg/L)		Removal Efficiency	Concentration (mg/L)		Removal Efficiency
Treatment	Algae	Initial	Final	(%)	Initial	Final	(%)
10% dairy wastewater	*S. dimorphus*	3.12±0.32	0.52±0.0.14	83.2	6.88±0.40	1.17±0.24	82.9
	C. vulgaris	3.12±0.32	2.99±0.01	4.1	6.88±0.40	5.45±0.59	20.8
	Mix	3.12±0.32	1.07±0.01	65.7	6.88±0.40	1.06±0.31	84.6
25% dairy wastewater	*S. dimorphus*	7.69±0.85	2.48±0.19	67.7	16.39±1.51	12.78±2.42	22.0
	C. vulgaris	7.69±0.85	3.43±1.49	55.3	16.39±1.51	14.42±1.06	12.0
	Mix	7.69±0.85	2.25±0.67	70.7	16.39±1.51	7.087±1.35	56.8

Table 6. TSS and VSS removal efficiencies.

		TSS			VSS		
		Concentration (mg/L)		Removal Efficiency	Concentration (mg/L)		Removal Efficiency
Treatment	Algae	Initial	Final	(%)	Initial	Final	(%)
10% dairy wastewater	*S. dimorphus*	140±30	110.0±20	21.4	113.33±32.15	73.33±20.82	35.3
	C. vulgaris	140±30	123.33±11.55	11.9	113.33±32.15	106.67±5.77	5.9
	Mix	140±30	46.67±5.77	66.7	113.33±32.15	36.67±5.77	67.6
25% dairy wastewater	*S. dimorphus*	140±45.83	93.33±23.09	33.3	123.33±25.17	56.67±20.82	54.1
	C. vulgaris	190±62.45	120±17.32	36.8	126.67±30.55	83.33±20.82	34.2
	Mix	220±14.14	105±35.36	52.3	125±21.21	60±42.43	52.0

maximum allowable concentrations of these nutrients from wastewater treatment plants. Hence, algal treatment could provide an environmentally sustainable alternative to conventional wastewater treatment systems which are highly energy intensive.

One of the goals of wastewater treatment is the reduction of total suspended solids (TSS) and volatile suspended solids (VSS). As expected, the initial values for TSS and VSS increase with increasing concentration of dairy manure wastewater. Highest reductions in TSS and VSS were achieved for mixed coculture at 10% dairy manure concentration. However, when normalized with respect to the biomass concentrations, highest TSS reduction was achieved with the *C. vulgaris* cultures while *S. dimorphus* had highest VSS reduction for both concentrations of dairy manure (table 7). The removal of NO_3^--N and PO_4^{-3}-P from manure effluent was similarly influenced by both manure concentration and algae biomass density (table 7) (Pizarro et al., 2002).

Based on the results, it is evident that the strains *S. dimorphus* and *C. vulgaris* have different capacities for biomass production (*S. dimorphus* is higher), lipid accumulation (*S. dimorphus* is higher), chlorophyll accumulation (*C. vulgaris* is higher), TSS removal (*C. vulgaris* is higher), and VSS removal (*S. dimorphus* is higher). These capacities can be leveraged in mixed coculture to achieve higher treatment efficiencies compared to monocultures (fig. 4). Additionally, mixed cocultures also offer higher stability and robustness which is an added advantage when designing low-cost wastewater treatment system using algae. It is well known that algal growth and nutrient removal efficiency could be increased based on many factors such as algal species/strain selection, initial cell concentration, aeration, and retention time. Many authors reported that *S. dimorphus* and *C. vulgaris* are a common and effective species for the immobilization and nutrient removal purposes (Lau et al., 1997; Abdel Hameed, 2007). In this study the mixed coculture of algae

Table 7. Total chlorophyll accumulation, nitrate, phosphate, TSS, and VSS removal normalized by biomass growth.

Treatment	Algae	Total Chlorophyll Accumulation (mg/g biomass)	Nitrate Removal (mg/g biomass)	Phosphate Removal (mg/g biomass)	TSS Removal (mg/ g biomass)	VSS Removal (mg/ g biomass)
10% dairy wastewater	*S. dimorphus*	7.20	10.65	23.39	122.97	163.96
	C. vulgaris	26.24	2.46	27.70	323.37	129.35
	Mix	9.35	6.21	17.64	282.86	232.35
25% dairy wastewater	*S. dimorphus*	27.96	11.95	8.27	107.04	152.91
	C. vulgaris	42.29	14.53	6.73	239.03	147.97
	Mix	23.34	10.23	17.51	216.40	122.31

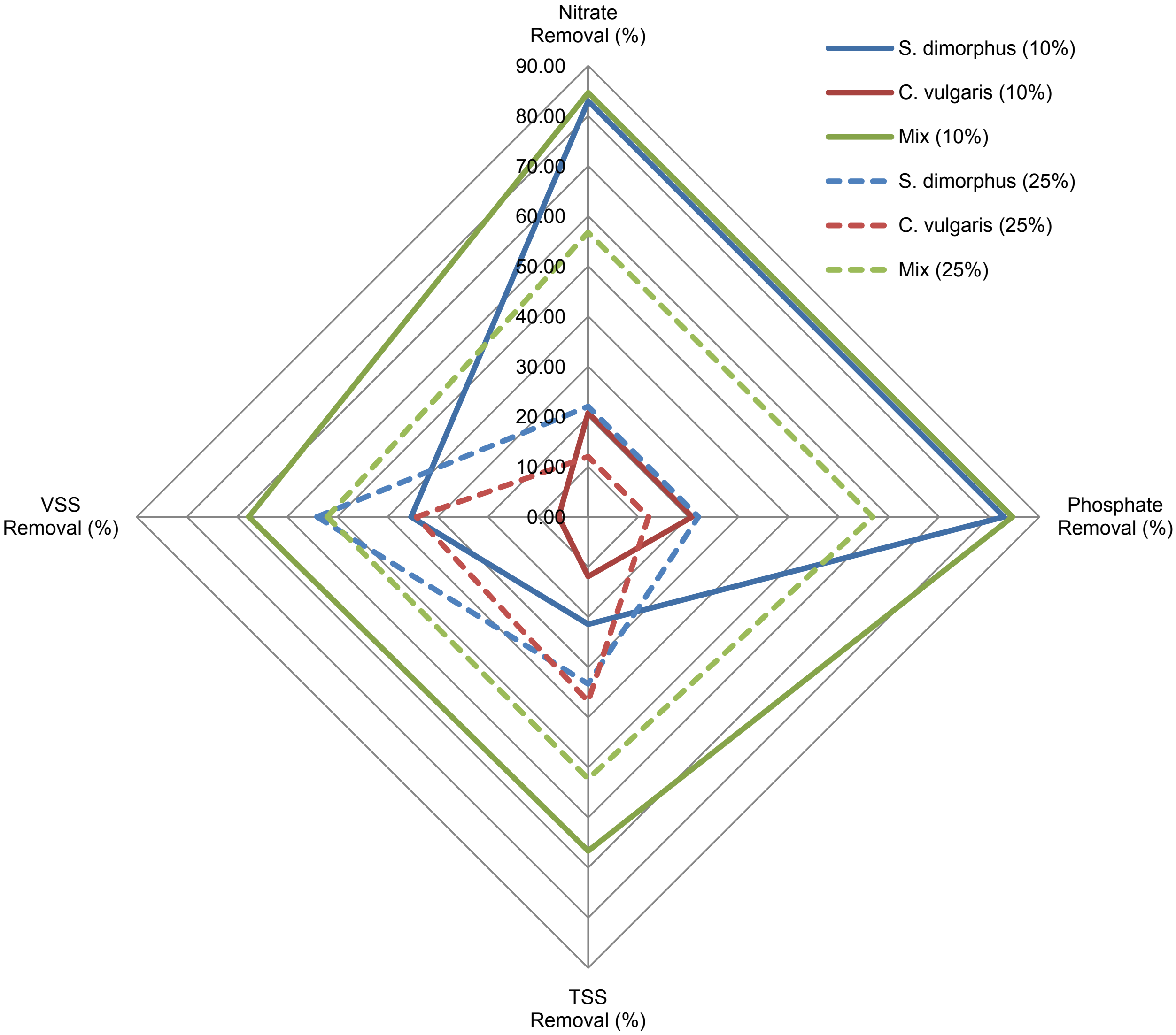

Figure 4. Removal efficiencies of nitrate, phosphate, TSS, and VSS.

strains *S. dimorphus* and *C. vulgaris* were found to be more efficient in removal of nutrients from dairy manure.

CONCLUSION

Two strains (*C. vulgaris* and *S. dimorphus*) of common fresh water algae and their mixed coculture were used to treat raw unsterilized dairy manure at two different concentrations. It was found that mixed coculture had higher biomass growth, specific growth rate, and removal efficiency of nitrogen, phosphorous, and TSS for the 25% dairy wastewater. The results were similar for 10% dairy wastewater except for the specific growth rate and nitrogen removal efficiency which were higher for the *S. dimorphus* monoculture. Based on these results, it could be stated that mixed coculture of algae offer unique advantages such as increased stability of cultures, increased treatment efficiencies, robustness, and possibly increased stability of the cultures compared to monocultures.

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