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Growth kinetics and purifying performance of *A. Platensis* in stirring-free culture based on brewery effluent's supernatant

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ABSTRACT

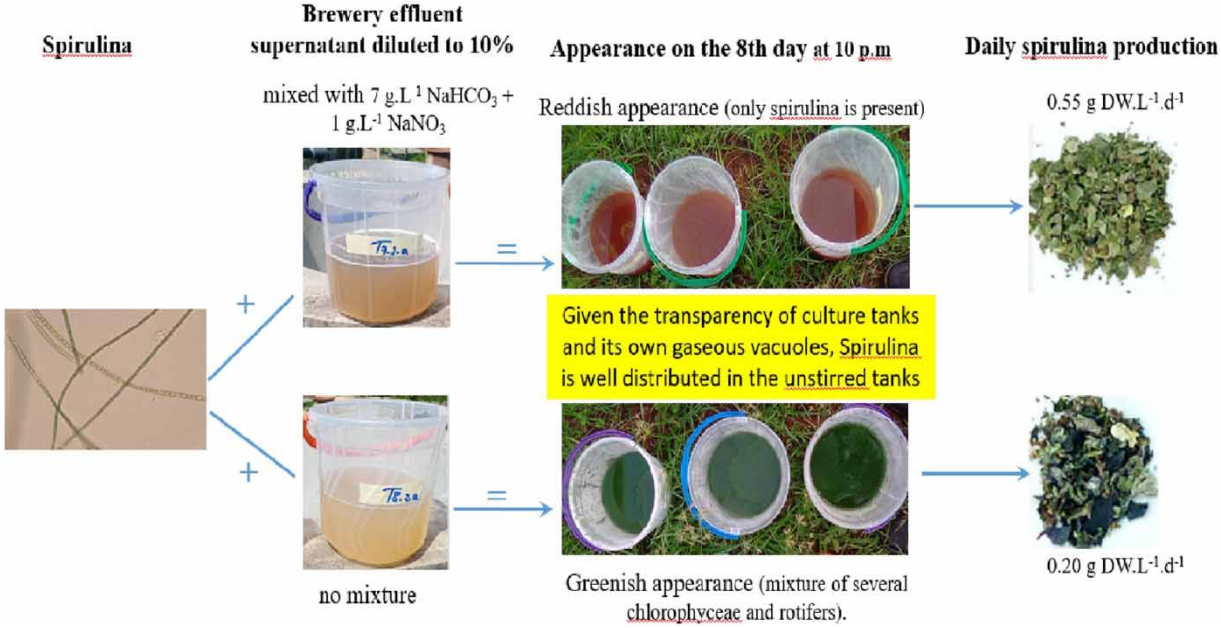
Due to costs of setting up and operating electrical stirring systems to keep algae in suspension and exposed to light, cultivation of monospecific algae is poorly expanded in developing countries. However, some algal species, such as *Arthrospira platensis*, are equipped with gaseous vesicles that allow them to stay afloat and increase their exposure to light. In this study, we investigated in an unstirred outdoor environment, its growth kinetic and purifying performance in a brewery effluent-based media. Batch cultures were carried out in three experimental treatments and evolution of physicochemical and growth parameters were monitored. Then its contribution to depollution was determined. Results show that optimal conditions for producing *A. platensis* include the culture tank transparency, the effluent dilution (i.e. 10%), and the culture media amendment with sodium bicarbonate and sodium nitrate. The average productivity recorded reached 0.55 g DW·L^{-1}·d^{-1} during the exponential growth phase, while preserving culture from contamination. COD and total nitrogen concentrations were reduced to 32.5 and 64.91%. Such results open up prospects for low-cost production of certain algae, in transparent and relatively high barrels, thus breaking the classic barriers related to shallow basin depth and mechanical agitation traditionally considered as critical to the success of algal production.

Key words: *Arthrospira platensis*, brewery effluent, transparent tank, valorization, wastewater treatment

HIGHLIGHTS

- These results open up prospects for low-cost production of certain algaes such as spirulina, in transparent and relatively high barrels, thus breaking classic barriers related to shallow depth of the basins and mechanical agitation traditionally considered in order to succeed in algal production.
- Furthermore, valorisation of agri-food industrial effluent contribute to lower production cost and depollute the wastewater.

GRAPHICAL ABSTRACT



INTRODUCTION

Algal cultures in outdoor environments are generally carried out in high-rate algal ponds (HRAP), which are shallow agitated basins (Borowitzka 2005). In such ponds the shallow depth (15–30 cm) maximizes algae exposure to light, critical for the photosynthesis. Moreover, the agitation prevents algae from settling. However, besides the technicality necessary to successfully agitate the system at about 30 cm·s^{-1} (Borowitzka 2005), operating HRAP is costly given the investment costs and operating costs related to power consumption and maintenance. These aspects make the technology unaffordable to small fish farmers in developing countries. Algal species including *Athrospira platensis,* commonly known as spirulina, have gas vesicles which allow them to stay afloat naturally and increase their exposure to light without requiring stirring (Tomaselli 1997). In this context, this work aims at assessing productivity kinetics, and water purifying capability of *A. platensis* in unstirred brewery effluent-based media in outdoor environment.

MATERIAL AND METHOD

Scope of the study

Experiments were carried out at the Wetland Research Laboratory of the University of Abomey-Calavi, Benin. In this laboratory, research themes focused on fish farming diversification and its development based on unconventional resources such as biomasses produced from recovering agri-food industrial effluents. The overarching purpose of this study is to contribute to the profitability of fish farming in developing countries through low-cost productions of proteins sources and other nutrients essential in fish farming.

Determination of nutritive potentials of brewery effluent's supernatant

The nutritives potential of the effluent supernatant in our study was determined by comparing its characteristics according to Liady *et al.* (2020) to those of the modified spirulina medium (Schlösser 1994, cited by Andersen *et al.* 2005). Characteristics of the brewery effluent supernatant and those of the Schlösser medium are presented in Table 1.

Liady *et al.* (2020) reported that nitrate and phosphate concentrations in brewery effluent supernatants similar to that used in this study followed a Gaussian distribution. The parametric student t-test was used to compare mean values of these elements in the supernatant and those of Schlösser's medium.

Table 1 | Comparison of brewery effluent supernatant with Schlösser's medium

Parameters		SS (g/L)	$N_{organic}$ (mg/L)	$P_{organic}$ (mg/L)	COD (g d'O_2/L)	BOD_5 (g d'O_2/L)	N-NO_3 (mg/L)	P-PO_4^{3+} (mg/L)	N-NH_3 (mg/L)	pH
Brewery effluent supernatant (Liady *et al.* 2020)	Average	48.23	2,349.56	121.89	231.37	7.59	4.33	352.13	357.78	4.5
	Standard deviation	23.42	1,312.99	45.96	114.57	2.06	2.02	115.3	148.52	0.2
Schlösser's medium		0	0	0	–	–	411	88	0	9.2

Mother culture used

Two mother cultures left outdoors for several weeks were used to inoculate experimental cultures. The first one consists of a culture on brewery effluent's supernatant amended with sodium bicarbonate (7 g·L^{-1}) and sodium nitrate (1 g·L^{-1}). It was used for seeding brewery effluent treatments. The second one consists of a culture on the modified spirulina medium (Schlösser 1994, cited by Andersen *et al.* 2005).

Harvesting spirulina in mother cultures

Because of their gaseous vesicles (Tomaselli 1997), spirulina cannot be harvested by centrifugation. The addition of certain salts can maximize the harvest of spirulina after flotation of its filaments (see for example Kim *et al.* 2005). However, this technique was not used in this study to avoid interference with the reagents used.

In this study, spirulina were harvested through three stages: before starting the experiment, each mother culture media was transferred to a new tank; it was cleared off from its flocs by filtration through a 200 μm mesh size's sieve; and finally, the filtrate was filtrated again through a 50 μm mesh size plankton net in order to concentrate the spirulina by separating it as well as possible from the initial culture medium.

Seeding dose

Seeding dose of spirulina in the experimental culture (hereafter referred to as child culture) was determined on the 10% diluted supernatant. A known volume (V_m) of known concentration of the mother culture medium (C_m) was put into a given volume of culture medium at 10% brewery effluent supernatant (V_f') until the appearance of a pale green coloration. From one treatment to another, and depending on the spirulina concentration in the mother culture medium, the final concentration of spirulina (Cf) was calculated through the following Equation (1):

$$V_m = \frac{C_f * V_{f'}}{C_m - C_f} \tag{1}$$

where V_m is the volume of the mother crop to be seeded in a given child culture; C_m is the concentration of the mother culture; C_f is the initial concentration to be achieved for the child culture at the start of the experiment; V_f' is the initial volume of the child culture before its seeding with V_m (note that the final volume of the child culture corresponds to $V_f' + V_m$).

Experimental and control cultures

The brewery effluent used came from SOBEBRA. Before the experiment, it was left to settle for 48 hours. The supernatant was then filtered through a 50 μm mesh size plankton net, and diluted to 10% to constitute the basic medium of the experimental cultures. Two experimental treatments were considered: one amended with sodium bicarbonate (7 g·L^{-1}) and sodium nitrate (1 g·L^{-1}) (T7), the second unamended (T8). These two experimental effluent treatments were then seeded with the first mother culture. A corrective treatment consisting of the supernatant diluted to 10% but not seeded (B) was carried out under the same conditions to correct the biomass of spirulina in the experimental tanks. Finally, a control treatment (T0) grown on the modified spirulina medium (Schlösser 1994, cited by Andersen *et al.* 2005) was prepared and seeded with the second mother culture to compare spirulina's growth rate in the effluent. The treatments T7 and T8 were repeated four times; T0 and B were repeated three times. The initial volume of each of the 14 experimental units was liters. All cultures were carried out in batch mode, in transparent buckets 20 cm high and 18 cm in average diameter. During the experiment, all the buckets were covered with mosquito nets (Figure 1).

Figure 1 | Experimental device used.

Monitoring spirulina growth

After seeding, the initial concentration of suspended solids (SS) was determined in T7 and T8. Suspended solids in those treatments consisted of spirulina and inert matter ($Ci_{(\mathrm{effl}+\mathrm{spir})}$). In T0 where it consists of spirulina and in B (corrective treatment) they only consisted of inert matter ($Ci_{\mathrm{effl\_t0}}$). The seeded spirulina biomass ($C_{ispir;to}$) was estimated as follows (Equation (2)):

$$Ci_{spir;t0} = Ci_{(effl+spir);t0} - Ci_{effl\_t0} \tag{2}$$

During the experiment, spirulina biomass was monitored every 2 days using samples collected from each child culture. Sampling was carried out after homogenization of the culture. The sample was dried at 70 °C in an oven (New life, model NL-9023) after filtration on glass microfiber filters (VWR1516-0875). Spirulina concentration ($C_{\mathrm{spir\_t}}$) was determined by subtracting $C_{(\mathrm{effl}+\mathrm{spir})\_t}$ from C_{effl_t} (Equation (3)):

$$C_{spir\_t} = C_{(effl+spir)\_t} - C_{effl\_t} \tag{3}$$

In addition, observations were systematically made under a tri-ocular microscope (BIMICRO) after weighing, to assess any potential contamination of the culture.

Determination of generation times

The exponential growth phase of each culture was identified through plotting, and the growth rates during this phase (μ) were determined, from the slope of line Equation (4):

$$\ln\left(\frac{Cspir_t}{Cspir_{t0}}\right) = \mu * t \tag{4}$$

Knowledge of these growth rates made it possible to calculate generation time (g_t) for each of the experimental unit as follows (Equation (5)).

$$g_t = \frac{\ln(2)}{\mu} \tag{5}$$

Productivity estimation

The productivity (p) of each culture was calculated during the exponential growth phase is as follows (Equation (6)):

$$p = \frac{(C_{spir\_t} - Ci_{spir;t0})}{t - t_0} \tag{6}$$

Monitoring of the physico-chemical parameters

Various physicochemical parameters including pH, temperature and conductivity were monitored every 2 days at 13:00 using a multiprobe (Hanna HI 991301).

Determination of the contribution to effluent purification

The contribution of *A. platensis* to effluent purification was assessed quantitatively in T7 treatment based on the calculations of COD, BOD, N_{TK} and P_{total} abatement rates of samples filtered before and after cultivation (Equation (7)):

$$Abatement\ (X) = \frac{C_i(X) - C_f(X)}{C_i(X)} \tag{7}$$

where $C_i(X)$ and $C_f(X)$ refer to the initial and final concentration of parameter X (COD, BOD, N_{TK} or P_{total}) in filtered brewery effluent.

COD, BOD, NTK and P_{total} measurements were carried out in accordance with Rodier *et al.* (2009).

Data processing

All statistical analyses were carried out using Statistica software. Growth rates were determined using linear regression by adjusting exponential phase data to a linear model without intercepts. They were then compared using a nonparametric analysis of variance (ANOVA) (i.e. the Kruskal-Wallis test). The statistical significance threshold was 5%. Effects of treatment, time, and time and treatment interaction on produced biomass were studied using a repeated-measures ANOVA after satisfaction of the sphericity hypothesis (Mauchley Sphericity Test). When these assumptions were not verified, a general linear model (GLM) was used. Multiple comparisons were made using Tukey's HSD test ($\alpha = 0.05$).

RESULTS

Nutritive potential of the brewery effluent supernatant

The effluent was poorer in nitrate than the Schlösser medium but was richer in orthophosphates, organic nitrogen and organic phosphorus (Table 2). These organic forms, whose concentrations are very important, could be made available after mineralization.

Evolution of physico-chemical parameters

Temperature evolution followed the same trend in all treatments. Average temperatures recorded were 36.17, 38.00 and 37.61 °C, respectively in the T0, T7 and T8 treatments. No significant difference ($p > 0.05$) was noted either between the average daily temperatures recorded over the period independently of the treatments (Table 3) or between average daily temperatures recorded in the different treatments (Table 4). Unlike temperature, pH and conductivity did not follow the same trend in all treatments. Significant differences ($p < 0.05$) were noted between average daily pH, average daily conductivities (Table 3), between average pH of different treatments and between average conductivities of different treatments (Table 4).

The average pH and conductivity recorded for T0, T7 and T8 were 9.76 and 20.00, 9.22 and 10.53 and 7.86 and 3.07 $mS{\cdot}cm^{-1}$, respectively.

Evolution of spirulina biomasses in different treatments

All cultures started their exponential growth without observing any latency. Then they developed similarly to reach their stationary phase from the 4th day into the culture in the effluent-based cultures and from the 6th day into the culture in the Schlősser medium (Figure 2 and Table 5). The maximum production was achieved on the 4th day of cultivation in all brewery effluent treatments, with mean values equalling $2.63 \pm 0.17\ g{\cdot}L^{-1}$ in the amended effluent (T7) and $1.05 \pm 0.25\ g{\cdot}L^{-1}$ in the unamended effluent (T8). In the Schlősser medium (T0) the mean value was $1.6 \pm 0.1\ g{\cdot}L^{-1}$. Significant

Table 2 | Comparisons of nutrient potential of the effluent's supernatant to those of Schlösser medium

Parameter	Mean	Standard deviation	N	Calculated t-value	Reference value (Schlösser medium)	Critical t-value	df	p
$N-NO_3^-$ ($mg{\cdot}L^{-1}$)	4.33	2.02	27	1,046.1	411	1.706	26	0.000
$P-PO_4^{3-}$ ($mg{\cdot}L^{-1}$)	352.13	115.30	27	11.90	88	1.706	26	0.000

Table 3 | Comparison of physico-chemical parameters

Parameter		Sum of squares	Df	Mean squares	F	p
Temperatures	Intercept	99.05	1	99.1	9.25	0.00
	Time (day)	37.94	1	37.9	3.54	0.07
	Treatment	30.56	2	15.3	1.43	0.25
	Error	546.13	51	10.7		
pH	Intercept	4.09	1	4.09	47.8	0.000
	Time (day)	8.60	1	8.60	100.6	0.000
	Treatment	34.57	2	17.29	202.2	0.000
	Error	4.36	51	0.09		
Conductivity $mS{\cdot}cm^{-1}$	Intercept	0.01	1	0.01	0.05	0.82
	Time (day)	1.09	1	1.09	6.09	0.02
	Treatment	2458.80	2	1229.40	6877.26	0.00
	Error	9.12	51	0.18		

Table 4 | Multiple comparison of average physicochemical characteristics

Treatment	T0	T7	T8
Temperature (°C)	36.17 ± 0.92^{a}	38.00 ± 0.65^{a}	37.61 ± 0.80^{a}
pH	9.76 ± 0.05^{a}	9.22 ± 0.07^{b}	7.87 ± 0.17^{c}
Conductivity ($mS{\cdot}cm^{-1}$)	20.00 ± 0.00^{a}	10.53 ± 0.15^{b}	3.07 ± 0.06^{c}

Treatments with the same letters are not statistically different ($\alpha = 0.05$).

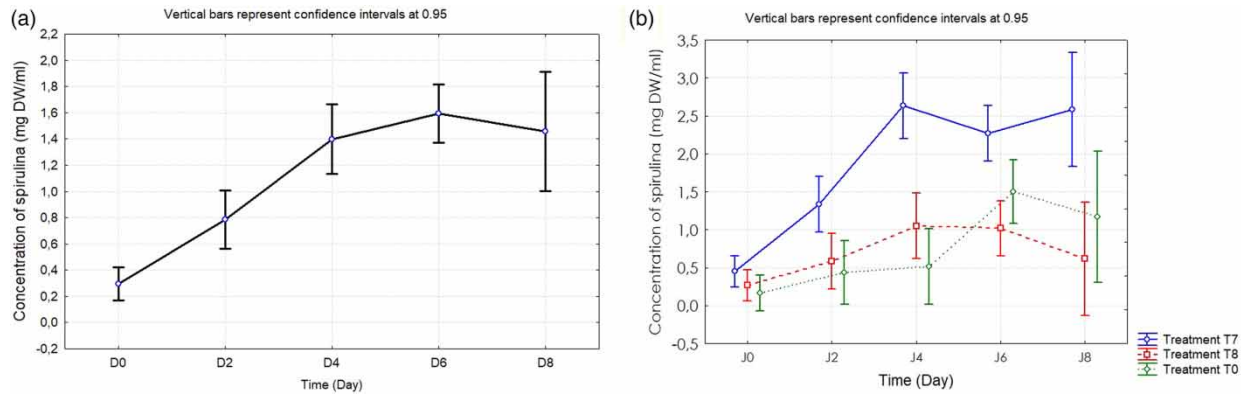


Figure 2 | Evolution of average spirulina concentrations: (a) regardless of treatments; (b) for each of the three treatments.

differences ($p < 0.05$) were found between average daily spirulina concentrations up to the 4th day (Table 5). From the 4th day till the 8th day there were no statistical differences ($p > 0.05$; Table 5).

Microscopic observations made in relation to culture contamination showed no contamination in T0, and contamination by rotifers in T7 and T8 (lower contamination in T7 compared to T8).

Generation time of *A. platensis* in the different treatments

Table 6 presents the calculated generation times. The generation time in the three treatments was not statistically significant ($p > 0.05$).

Table 5 | Comparison of average daily spirulina concentrations

Time (day)	J0	J2	J4	J6	J8
Daily siprulina concentration (mg DW·mL^{-1})	0.31 ± 0.06^{a}	0.82 ± 0.15^{b}	1.48 ± 0.30^{c}	1.60 ± 0.19^{c}	1.48 ± 0.32^{c}

Treatments with the same letters are not statistically different ($\alpha = 0.05$).

Table 6 | Generation time of spirulina in different treatments

Treatment	Number of repetition	Average generation time (day)	Confidence −95%	Confidence +95%	Standard error
T7	4	1.54	0.80	2.28	0.23
T8	4	2.87	−1.44	7.19	1.36
T0	3	1.98	0.48	3.48	0.35

Productivity of *A. platensis* in the different treatments and contribution to effluent purification

During the exponential growth phase, the average spirulina productivity was noticeably higher in treatment T7 (0.55 ± 0.05 g DW·L^{-1}·d^{-1}) than in T0 (0.22 ± 0.01 g DW·L^{-1}·d^{-1}) and T8 (0.20 ± 0.04 g DW·L^{-1}·d^{-1}). For the latter two there was no statistical difference ($p > 0.05$) (Table 7).

During the monitoring period, the appearance of sun-dried spirulina varied from dark bright green in T0 to less dark green in brewery effluent treatments (Figure 3).

Regarding the capability to purify the brewery effluent, the concentrations of COD and total nitrogen were reduced to 32.5 and 64.91%, respectively, in T7 after 6 days into the culture.

DISCUSSION

Dilution ratio

The dilution ratio used in this work was 10% compared to the ratio (20%) used in Lu *et al.* (2017). This difference was justified by the fact that the supernatant used in this work was doubly loaded (23,137 ± 11,457 mg COD·L^{-1}). In Lu *et al.* (2017) the organic load was 10,120 ± 233 mg COD·L^{-1}.

Evolution of physico-chemical parameters during the experiment

The similarity of temperature trends in the three treatments indicated that they were subjected to similar temperature conditions. The statistically significant differences for pH and conductivity found in this study can be explained by differences in chemical conditions between treatments, particularly the differences in alkalinity.

Indeed, there seems to be a good linear correlation between sodium bicarbonate intakes and observed conductivity and pH values. High values were observed in T0 which initially experienced an intake of 13.61 g $NaHCO_3$.L^{-1}, followed by T7 which experienced 7 g·L^{-1}, and T8 which experienced no intake and whose initial pH was slightly acidic (6.63 ± 0.18).

Spirulina productivity

The relatively high density of rotifers observed in T8 compared to T7 does not explain the significant difference noted between productivities in these two treatments. Indeed, Mitchell & Richmond (1986) have shown that rotifers rather preserve spirulina crops against contamination by unicellular algae by feeding on them. The difference in productivity found between these two treatments can be explained by alkalinity (here related to bicarbonates) and concentrations in nitrates. Bicarbonates

Table 7 | Comparison of average daily spirulina concentrations

Treatement	T7	T8	T0
Average daily spirulina concentrations (g DW·L^{-1}·d^{-1})	0.55 ± 0.05^{a}	0.20 ± 0.04^{b}	0.22 ± 0.01^{b}

Treatments with the same letters are not statistically different ($\alpha = 0.05$).

Figure 3 | Aspects of spirulina produced after sun drying (from left to right respectively, culture on Schlösser medium, on amended effluent and on unamended effluent).

improve the availability of mineral carbon sources and create selective conditions that prevent the proliferation of other microorganisms by raising the alkalinity of the medium. This was confirmed by the absence of rotifers in the T0 medium in our study. Nitrates provide mineral nitrogen (whose brewery effluent is poor) to satisfy the needs of spirulina. The productivity observed in T7 in this study was not significantly different from that obtained in Lu *et al.* (2017) in the pretreated brewery effluent, nor from those obtained respectively by Raoof *et al.* (2006) cited by Lu *et al.* (2017) who worked on the modified Zarrouk medium (Table 8). However, it was higher than those obtained in Volkmann *et al.* (2008), Jung *et al.* (2014), Salla *et al.* (2016), and da Rosa *et al.* (2016) (all cited by Lu *et al.* (2017)).

Contribution of *A. platensis* to effluent purification

The purification performance observed in this study can be explained by the same mechanisms as those observed in the last basins of extensive wastewater treatment systems such as the natural lagoon that algae colonize naturally.

In these basins where organic loads are low and mineral loads are high, algae maintain a symbiotic relationship with other microorganisms such as bacteria, consuming nutrients from the mineralization of organic matter and providing oxygen favorable to these microorganisms for their respiratory activities. The reduction of the organic load is attributable to microorganisms such as bacteria that are likely to grow in this environment, despite the adversity related to the alkalinity of the environment.

Table 8 | Productivity and duration of cultivation of spirulina grown under different conditions

Medium	Production (g DW·L^{-1})	Duration (d)	Productivity (g DW·L^{-1}·d^{-1})	Source
T7	2.18 ± 0.21	4	0.55	This study
T0	1.34 ± 0.06	6	0.22	This study
T8	0.78 ± 0.16	4	0.20	This study
Pretreated brewery effluent	1.56	5	0.31	Lu *et al.* (2017)
Modified Zarrouk medium	0.57	6	0.31	Raoof *et al.* (2006) cited by Lu *et al.* (2017)
Paoletti medium	2.5	23	0.10	Volkmann *et al.* (2008) cited by Lu *et al.* (2017)
Zarrouk medium with monoethanolamine	1.2	12	0.11	da Rosa *et al.* (2016) cited by Lu *et al.* (2017)
Zarrouk medium with whey protein	1.5	16	0.10	Salla *et al.* (2016) cited by Lu *et al.* (2017)
Zarrouk medium with shell and soil extract	2.2	14	0.09	Jung *et al.* (2014) cited by Lu *et al.* (2017)

Table 9 | Depollution performances of algaes

Algal specie	Culture		Abatment rate (%)			
	Duration (d)	Condition	COD	N_{Total}	P_{Total}	Reference
Chlorella vulgaris	20	Laboratory culture on brewery effluent undiluted and diluted to 1:2 and 1:1 (v/v)	14.6	63	28	Raposo *et al.* (2010)
Scenedesmus obliquus	13	Laboratory culture on synthetic brewery effluent	57.5	20.8	N.A	Mata *et al.* (2012)
Spirulina sp	5	Laboratory culture on centrifuged 20% diluted and enriched brewery effluent	75.2	78.3	97.4	Lu *et al.* (2017)
A. platensis	6	Outdoor culture on 10% diluted and enriched, brewery effluent	32.5	64.91	–	This study

Some depollution performances by algae are given in Table 9. The difference between organic matter (COD) yield found (lower than that reported in Lu *et al.* 2017) could be explained by differences in accounting methods. In their method, Lu *et al.* (2017) considered the contribution of the preliminary stage of anaerobic digestion of the effluent. This was not the case in our study.

Reductions of pollutant loads observed in our growing conditions are similar to those reported in Raposo *et al.* (2010) for total nitrogen on production of *Chlorella vulgaris* in laboratory condition, though during a longer time than in this study (Table 9).

CONCLUSION

We investigated the growth kinetic, productivity, and purifying performance of *A. platensis* in an unstirred brewery effluent-based media in an outdoor environment. Results indicated that given their gas vesicles which allow them to stay afloat naturally and increase their exposure to light, *A. platensis* can be successfully used as a low-cost alternative of electrical stirring systems for cultivating monospecific algae. Valuing agri-food industrial effluent in certain outdoor settings contribute both to lower the production cost and depollute. Such results highlight the potential for low-cost production of certain algae in transparent and relatively high barrels, thus breaking the classic barriers traditionally considered as critical to the success of algal production.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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