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A high rate ponding unit operation linking treatment of tannery effluent and *Arthrospira* (*Spirulina*) biomass production. 1: Process development

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ARTICLE INFO

Article history:
Received 9 January 2012
Received in revised form
4 January 2013
Accepted 7 January 2013
Available online 14 February 2013

Keywords:
Spirulina
Biofuels
Animal feed
Tannery
Wastewater
Waste stabilization ponds

ABSTRACT

Effluent from the production of wet blue leather has been shown to support the substantial growth of *Arthrospira* biomass in tannery waste stabilization ponds, which is of interest in its use both in animal feeds and biofuels production. Here we report process development investigations which were undertaken in photobioreactor and outdoor high rate pond pilot studies. Biomass productivities of 16 t ha^{-1} yr^{-1} (dry mass) were measured which compares broadly with yields reported for *Arthrospira* cultivated in other complex media. The specific growth rate $\mu = 0.05$ d^{-1} was somewhat lower than reports for the mixotrophic cultivation of *Arthrospira* in defined media studies. This system operates under ammonia control and may be relieved by recirculation of alkaline waters which accumulate in these waste stabilization ponds. A substantial difference in total nitrogen and phosphorus removal between the experimental and theoretical yields due to biological activity suggests stripping may account for the largest fraction of ammonia removal, and precipitation for phosphate removal, in this operation. Heavy metal contamination may be a problem with biomass production in industrial effluents and pretreatment of the tannery effluent in a primary facultative pond was shown to substantially reduce the heavy metal load. Process kinetic values were derived and have been used for the design and construction of a full-scale *Arthrospira* production operation using tannery effluent growth media, which is reported in a follow-up study.

1. Introduction

The resurgence of interest in microalgal biofuels development [1,2], has focused attention on the use of wastewaters as a means of reducing the cost of growth media formulation [3]. This remains one of the major production inputs determining the profitability of the biofuels enterprise [4], and the linkage of waste treatment and microalgal biomass production provides an opportunity to achieve reductions in both operational and capital costs [5].

Waste stabilization ponds (WSP) have been widely used in the treatment of domestic and industrial wastewaters [6] and their application in tannery effluent treatment, as zero-discharge systems, provides one of few treatment options available for leather production in highly water stressed areas [7,8]. While the appearance of massive near mono-species blooms of *Arthrospira* (*Spirulina*) have been described in these systems [9], little has been reported on factors regulating this growth in tannery effluents and these blooms remain unpredictable and unreliable in their appearance. The role of

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http://dx.doi.org/10.1016/j.biombioe.2013.01.025

microalgae in the successful operation of WSP has been well-described [10,11] and controlled use of *Arthrospira* growth could be of importance in the management of the tannery WSP, and particularly in the control of odour nuisance, to which they are prone [12].

The current investigation follows a previous evaluation of the potential use of tannery effluents as production media for the mass culture of *Arthrospira* biomass [13]. Here it was shown that in effluent from a wet-blue tanning operation, *Arthrospira* growth is under the direct control of the ammonia concentration in the growth medium. This indicated that an effective mass culture strategy in this medium would require the determination of a maximum effluent loading rate that operates as a function of the optimized ammonia removal rate. Determination of these kinetics under controlled conditions could provide a basis for the development of a full scale unit operation linking the treatment of tannery effluent and *Arthrospira* biomass production. This potential has been investigated in photobioreactor and outdoor pilot plant studies and is reported here.

2. Materials and methods

The site for this study was a tannery WSP located in the Western Cape Province, South Africa, and received about 460 $m^3\ day^{-1}$ effluent from the processing of 1500 hides to wet blue leather daily. Combined tannery wastewaters passed through physico-chemical pre-treatment, following process stream segregation and including sulfide oxidation, balancing and aeration and flocculent-assisted sedimentation with removal of solids, before discharge to the WSP cascade. The medium used in the study was drawn at this point and is referred to hereafter as tannery effluent. The *Arthrospira* strain used in the study was sourced from the tannery WSP. It was provisionally identified as *Arthrospira platensis* and was previously shown to grow mixotrophically in this medium [13].

A 5L New Brunswick Bioflo 111 fermenter was configured as a photobioreactor with an array of 50 cm cool white fluorescent tubes arranged around its circumference. A 10% inoculum of the tannery WSP *Arthrospira* isolate was used for reactor start-up in a 5% tap water dilution of tannery effluent (Table 1).

Once photosynthetically generated dissolved oxygen (DO) reached 7 mg L^{-1}, feeding of the reactor commenced. The reactor was operated at 25 °C under 12 h dark/light cycle with illumination at 158 μmol $m^{-2}\ s^{-1}$, and in batch-fed mode with the addition of undiluted effluent at the equivalent of 3% of reactor volume daily. After the establishment of a stable system, the continuous-feed operation commenced at increasing rates of tannery effluent addition. Steady state was assumed for each loading rate after at least three replacements of reactor volume. Temperature, DO and pH were computer logged at 15 min intervals.

A pond water alkalinity recirculation strategy was evaluated in flask studies. Tannery effluent in 1L Ehrlenmeyer glass flasks was inoculated with *Arthrospira* culture sourced from the steady state photobioreactor, fed at the 5% loading rate. Alkaline recirculation water, sourced from the terminal pond in the study site WSP, was added to triplicate flasks at 5%, 15% and 25% by volume. Photosynthetic carbon fixation was measured and results reflect a mean of triplicates.

Table 1 – Analysis of the tannery effluent used in this study (standard deviation in brackets).

(mg L^{-1})	
Chemical oxygen demand	2474(1810)
Ammonia as NH_3	731(98)
Phosphate as PO_4	19(12.5)
Calcium	226(11)
Chloride	4048(202)
Sodium	3090(198)
Sulphate	364(43)
Sulfide	1192(112)
Total alkalinity (as $CaCO_3$)	525(49)
Dissolved oxygen	0.01(0.01)
Salinity (gL^{-1})	10(0.039)
pH	8.2(0.2)

Analyses of COD, nitrate, ammonia and phosphate followed [14]. Salinity was measured with an Atago refractometer. Chlorophyll a was measured following the method of Lichtenthaler [15]. Measurements of photosynthetic productivity used the [^{14}C]-sodium bicarbonate CO_2 fixation method modified by Oren [16]and sample measurement was undertaken in a Beckman LS3150T scintillation counter. Heavy metal and nutritional analyses were undertaken by the Animal and Poultry Science Laboratory, University of Kwa-Zulu Natal.

Kinetic values for the process were derived in a continuous-feed operation at steady state. The specific growth rate μ (d^{-1}) was derived from the dilution rate D (d^{-1}) with growth at steady state for each feed rate. Volumetric biomass productivity Q_X (mg $L^{-1}\ d^{-1}$) was calculated as the concentration of biomass removed from the reactor as a function of time. The volumetric removal rates for total nitrogen (as N) Q_{NT} (mg $L^{-1}\ d^{-1}$) and phosphate (as P) Q_P (mg $L^{-1}\ d^{-1}$) were calculated as the difference between the feed and the reactor concentration as a function of the daily loading rate. Experimental removal yields in the reactor for nitrogen and phosphate, Y^1_{NT} and Y^1_P (dimensionless) were calculated as the ratio of amounts removed to those present in the feed. Theoretical removal ascribed to biomass uptake Y^2_{NT} and Y^2_P (dimensionless) was calculated by material balances from the data of cell mass production using an average dry biomass composition for *A. platensis* of $C_{1.650}\ O_{0.531}\ N_{0.170}\ S_{0.007}\ P_{0.006}$ [17]. Total nitrogen removal was calculated according to $N_{rem} = N^{IN}_{NH3} - (NH_3 + NO_3)^{SOL}$ and assuming no significant further ammonification and zero level nitrate in the influent.

3. Results and discussion

The process development investigation of a mixotrophic *Arthrospira* HRP unit operation in the treatment of tannery effluent was based on the results of preliminary flask studies [13], and was undertaken through scale-up from photobioreactor to pilot plant studies which are described here.

3.1. Photobioreactor

In the start-up phase the photobioreactor was operated in batch-fed mode, COD levels were reduced from 2474 mg L^{-1} to around 250 mg L^{-1}, DO remained well above saturation levels