Bioresource Technology 148 (2013) 366–372

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Decolorisation of piggery wastewater to stimulate the production of *Arthrospira platensis*

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HIGHLIGHTS

- The dark color of piggery wastewater results in low growth rates of *Arthrospira*.
- Oxidation, positively charged flocculants, biopolymers or adsorbents can remove color.
- Color removal results in a doubling of *Arthrospira* growth rates.
- Decoloring methods that remove P result in P limitation and decrease in biomass.

ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form 29 August 2013
Accepted 31 August 2013
Available online 11 September 2013

Keywords:
Color removal
Piggery wastewater
Spirulina
Phosphate recovery
Microalgae

ABSTRACT

The aim of this study was to evaluate the potential of color removal methods for enhancing the growth rate and biomass yield of *Arthrospira* produced using piggery wastewater as a nutrient source. Color could be removed from the piggery wastewater by means of oxidation (H_2O_2–UV) or by means of positively charged flocculants (e.g., ferric chloride, magnesium hydroxide), biopolymers (chitosan, cationic starch) or adsorbents (hydrotalcite). Some methods remove not only color but also phosphate (e.g., hydrotalcite) while other do not affect phosphate concentrations (e.g., chitosan). Color removal using chitosan resulted in a doubling of initial growth rate and a 50% increase in final biomass yield of *Arthrospira* produced on piggery wastewater. Color removal using hydrotalcite resulted in a low biomass yield of *Arthrospira* due to phosphate limitation.

1. Introduction

To avoid eutrophication of surface waters by discharges of wastewater, nitrogen and phosphorus are removed during wastewater treatment, often at a considerable cost. Conventional wastewater treatment systems remove nitrogen and phosphorus from wastewaters but do not re-use them. In recent years, however, the costs of nitrogen and phosphorus for industrial or agricultural use have increased dramatically as a result of increasing energy prices or declining global reserves (e.g., Cordell et al., 2009). Therefore, there is a growing incentive to re-use nitrogen and phosphorus from wastewaters. Microalgae are considered to be a promising technology to do this, as microalgae can convert nitrogen and phosphorus into a biomass that can be used for energy, animal feed or even high-value products (Park et al., 2011; Cai et al., 2013).

The species of microalgae that is today produced on the largest scale worldwide is *Arthrospira platensis* (Vonshak, 1997). Compared to other microalgae, production of *Arthrospira* is relatively straightforward because cultures are resistant to contamination and the biomass can easily be harvested. Moreover, *Arthrospira* has an attractive biomass composition due to its high protein content (up to 60%), the presence of the essential fatty acid gamma-linolenic acid and the blue pigment phycocyanin. The biomass can therefore be used in a biorefinery context to yield both high and low value products (food, feed, pigments, cosmetics). Already in 1974, it was shown that *Arthrospira* can be cultivated using domestic wastewater as a source of nitrogen and phosphorus (Kosaric et al., 1974). Since then, *Arthrospira* has been used to remove and re-use nutrients from a variety of wastewaters, including piggery wastewater, dairy wastewater, olive-oil mill effluents and human urine (e.g., Olguín et al., 2003; Markou et al., 2012).

A problem with treatment of domestic wastewater using microalgae is the lack of available land near urban areas (Park et al., 2011). With treatment of wastewaters of agricultural origin this is usually not a problem as nearby agricultural lands can be converted into algal ponds. However, wastewaters of agricultural origin such as effluents from animal manure processing, from anaerobic digestion of plant biomass or from food or feed production are often dark in color, which is usually ascribed to humic

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http://dx.doi.org/10.1016/j.biortech.2013.08.165

substances (Brezonik and Arnold, 2011; Guo et al., 2012). This dark color causes light limitation of microalgae and therefore limits the rate of nutrient recovery from wastewater (De Pauw et al., 1980; Markou and Georgakakis, 2011). In many studies evaluating treatment of wastewater using microalgae, this light limitation is avoided by diluting the wastewater with pure water. In full-scale systems, this is unsustainable due to the large volume of water required. Therefore, it is desirable to pre-treat the wastewater to remove or reduce the color prior to nutrient recovery by microalgae.

Color removal is commonly applied in treatment of colored wastewaters (Crini, 2006). Color can be removed by oxidation (e.g., using hydrogen peroxide or sodium hypochlorite) or can be removed by flocculants, biopolymers or adsorbents (Robinson et al., 2001; Crini, 2006). So far, few studies have investigated whether color removal enhances microalgal growth on wastewaters. Blais et al. (1984) removed color from piggery wastewater through dialysis and noted a marked increase in growth rate of *Phaeodactylum* in seawater amended with dialysed wastewater. Markou et al. (2012) treated olive-oil mill wastewater with sodium hypochlorite to enhance productivity of *Arthrospira* but concluded that other approaches should be explored because the use of sodium hypochlorite raises environmental issues. When applying color removal methods to wastewater, care should be taken that color removal does not affect phosphate concentrations as some flocculants, biopolymers or adsorbents commonly used for color removal also remove phosphates. This should be avoided as it limits the re-use of phosphorus in the wastewater by microalgae.

The goal of this study was to enhance the productivity of *Arthrospira* on piggery wastewater by color removal from the wastewater. The first specific aim of this study was to select a color removal method that removes color from piggery wastewater but does not affect phosphate concentrations. The second specific aim was to demonstrate that color removal enhances growth rates of *Arthrospira* in piggery wastewater.

2. Methods

2.1. Piggery wastewater used in experiments

Piggery wastewater is a major source of nutrients worldwide and has a high nutrient load as well as a characteristic dark color (Olguin et al., 2004). The wastewater used in this study was the liquid fraction of piggery waste obtained after Single reactor system for High activity Ammonium Removal Over Nitrite (SHARON) treatment (Tikkal, Pittem, Belgium). Solids were separated from the raw piggery waste using centrifugation. The liquid phase was subjected to the SHARON treatment, which occurs in a single mixed tank with alternating aerated and anoxic phases. After the SHARON treatment, the wastewater is transferred to a decanter to remove the sludge by settling. The supernatant of this decanter was used in this study. The physico–chemical characteristics of the wastewater used in this study are shown in Table 1. The wastewater contained 352 mg L^{-1} NO_3^--N, 70 mg L^{-1} NH_4^+-N, 168 mg L^{-1} total P and 129 mg L^{-1} PO_4^{3-}-P, while the total organic carbon content was 1409 mg L^{-1}.

Color of wastewater is usually measured spectrophotometrically in the near ultra-violet range (200–380 nm; Weishaar et al., 2003). In this study, absorbance at 360 nm was used as a measure for color (cf. Grieve, 1985). In order to allow measuring the optical density without the need for diluting the samples, the wastewater was diluted 10-fold prior to the experiments. Color of the piggery wastewater as estimated from absorbance at 360 nm was about 59% reduced by the SHARON treatment, most likely due to partial degradation of humic substances (Guo et al., 2012).

Table 1
Physico-chemical characteristics of the wastewater after the SHARON treatment.

Parameter	
pH	8.4
NO_3^--N (mg L^{-1})	352
NH_4^+-N (mg L^{-1})	70
Total P (mg L^{-1})	168
PO_4^{3-}-P (mg L^{-1})	129
Total carbon (mg L^{-1})	2144
Total inorganic carbon (mg L^{-1})	735
Total organic carbon (mg L^{-1})	1409
Mg (mg L^{-1})	81
Ca (mg L^{-1})	105

2.2. Color removal experiments

A wide range of color removal methods that are commonly used in different industries were tested (Robinson et al., 2001; Crini, 2006). In all experiments, both the concentration of the reagents were varied as well as the pH. The concentration ranges selected for the experiments were determined based on exploratory experiments. All experiments were carried out in 100 mL beakers stirred by a magnetic stirrer. The color removal (*CR*; %) in the experiments was calculated as:

$$CR = (OD_i - OD_f)/OD_i \cdot 100$$

where OD_i is the initial optical density before treatment and OD_f is the final optical density after the treatment.

(i) H_2O_2–UV combination: A solution of 30% H_2O_2 was added to the wastewater. After stirring during 10 min, the wastewater was exposed to UV light (Philips TLD, distance of 5 cm) for 40 h prior to measurement of OD_f.

(ii) Metal flocculants (ferric chloride, aluminium sulphate): Before addition of the metal flocculants, the pH of the wastewater was adjusted to pH 4, 6 or 8 by addition of 0.5 M HCl or 0.5 M NaOH. Different concentrations of ferric chloride ($FeCl_3 \cdot 6H_2O$, 99%) or aluminium sulphate ($Al_2(SO_4)_3 \cdot 18H_2O$, 98%) were added. After 30 min of stirring, the solution was allowed to settle for 60 min prior to measurement of OD_f.

(iii) Coagulation by magnesium hydroxide: Magnesium hydroxide precipitates are used as a coagulant in lime softening (Randtke et al., 1982) and are also used for flocculating microalgae (Vandamme et al., 2012). Magnesium hydroxide precipitates were formed by addition of magnesium to the wastewater and increasing the pH above pH 10.5. Magnesium sulphate was added to the wastewater at a concentration of 1.5 and 5 mM and pH was adjusted to pH 10.5, 11 and 12 by addition of NaOH. OD_f was measured after 30 min of stirring followed by 60 min of settling.

(iv) Biopolymers (chitosan, cationi6c starch, xanthan gum, alginate): pH was adjusted to pH 4, 6 and 8 by addition of 0.5 M HCl or 0.5 M NaOH before addition of the biopolymers. Different concentrations of chitosan (from shrimp shells <1% insoluble matter), cationic starch (Greenfloc 120 cationic biopolymer), xanthan gum (from *Xanthomonas campestris*) or sodium alginate (alginic acid salt from brown algae) were added. After 30 min of stirring, the solution was allowed to settle for 60 min prior to measurement of OD_f.

(v) Adsorbents (hydrotalcite, bentonite, kaolin and diatomite): Before addition of the adsorbents, the pH was adjusted to pH 4, 6 and 8 by addition of NaOH or HCl. For each type of adsorbent, 5 different concentrations were tested (125, 250, 500, 1000 and 5000 mg L^{-1}). The OD_f was measured after 30 min of stirring followed by 60 min of settling. Because the suspended adsorbents interfered with optical