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Remediation of a mixture of analgesics in a stirred-tank photobioreactor using microalgal-bacterial consortium coupled with attempt to valorise the harvested biomass

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HIGHLIGHTS

- Algal-bacterial artificial consortium was used to degrade 3 analgesics in STPBR.
- The tested analgesics and their toxic metabolites were removed efficiently.
- 24 h daily illumination at short HRT (3 days) led to the best remediation results.
- The biomass showed rapid settleability and was harvested for further analysis.
- The biomass was rich in bioproducts, which could be used in various applications.

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ABSTRACT

An artificial microalgal-bacterial consortium was used to remediate a mixture of analgesics (ketoprofen, paracetamol and aspirin) in a stirred-tank photobioreactor. A hydraulic retention time (HRT) of 3 days supported poor treatment because of the formation of *p*-aminophenol (paracetamol toxic metabolite). Increasing the HRT to 4 days enhanced the bioremediation efficiency. After applying an acclimatization regime, 95% removal of the analgesics mixture, *p*-aminophenol and COD reduction were achieved. However, shortening the HRT again to 3 days neither improved the COD reduction nor ketoprofen removal. Applying continuous illumination achieved the best analgesics removal results. The harvested biomass contained 50% protein, which included almost all essential amino acids. The detected fatty acid profile suggested the harvested biomass to be a good biodiesel-producing candidate. The water-extractable fraction possessed the highest phenolic content and antioxidant capacity. These findings suggest the whole process to be an integrated eco-friendly and cost-efficient strategy for remediating pharmaceutical wastewater.

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1. Introduction

The consumption rate of pharmaceuticals and personal care products (PPCPs) is increasing worldwide. Although PPCPs are considered to be among environmental threats, their allowable limits in water discharges are not regulated by law (Santos et al., 2010). In particular, analgesics are the most emerging problem in terms of consumption rate, potential influence on human health and environmental impact (Wu et al., 2012). These pollutants may enter the environment during the manufacture processes, improper disposal of unused or expired drugs or even through domestic discharges of non-metabolized excreted drugs (Wu et al., 2012). Optimizing a suitable and cost effective biodegradation system could combat this emerging problem.

In this regard, several studies have been conducted on the occurrence and removal of mixtures of different classes of pharmaceuticals such as analgesics, antiseptics, natural estrogens, beta-blockers, lipid-lowering agents, antibiotics and psychiatric agents during regular municipal wastewater treatment (Nakada et al., 2006; Yu et al., 2006; Kasprzyk-Horden et al., 2009; Zorita et al., 2009; Jelic et al., 2011). These were continued recently, where Marcelino et al. (2016) have examined the aerobic and anaerobic degradability and toxicity of real pharmaceutical wastewater from industrial production of antibiotics. However, most of these studies assessed the levels of pharmaceuticals in the influents and

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effluents of wastewater treatment plants in different countries during the conventional activated sludge treatment method.

In a previous study, Reardon et al. (2002) reported that mixtures of compounds could affect microbial degradation due to inhibition by either competition or toxicity. Hence, biodegradation processes would preferably depend on the use of a microbial consortium rather than a single microorganism (Safonova et al., 2004). In this regard and over the last two decades, biological treatment of wastewater using microalgal-bacterial systems proved to be the most promising and cost-effective tool (Munoz et al., 2005; Essam et al., 2013). In these biological systems, microalgae mitigate CO_2 and produce O_2 and biomass (Wang et al., 2013). In addition, the generated biomass could be further applied in several fields such as animal feeding stuff and production of bioproducts for the medical, pharmaceutical and industrial use (Harun et al., 2010).

The approach of dual-purpose systems employing microalgal-bacterial combination to treat wastewater and produce valuable bioproducts has recently grasped considerable attention (Olguín, 2012). This approach provides an integrated biorefinery system, and is considered an attractive and cost-effective alternative to systems aiming solely to produce microalgal biomass to obtain biofuel and/or other bioproducts. Moreover, this approach adds value to treating wastewater, eliminates the need for fresh water resources for microalgal biomass production and reduces the cost of aeration needed for aerobic degradation of pollutants as the bacteria will get the required O_2 from the microalgae undergoing photosynthesis (Olguín, 2012).

In this perspective, to the best of our knowledge this is the first study to introduce the application of microalgal-bacterial system for the degradation of a mixture of some of the commonly used analgesics (paracetamol, aspirin and ketoprofen) in an artificial wastewater. Both of the treatment and detoxification efficiencies were monitored and optimized. The dual-purpose approach of pharmaceutical wastewater treatment and production of valuable bioproducts was attempted through harvesting and analysing the generated biomass to determine its main bioproducts and possible fields of applications.

2. Materials and methods

2.1. Microorganisms

An analgesics-tolerant microalgal strain (labelled as A_1) and an analgesics-degrading bacterial consortium (labelled as K_2) were used to construct an artificial microalgal-bacterial consortium (A_1K_2) at a ratio of 5: 1, respectively, according to Guieysse et al. (2002). These microorganisms were previously isolated and molecularly identified (Ismail et al., 2016). Briefly, the bacterial consortium K_2 was composed of four Gram negative bacteria: *Raoultella ornithinolytica, Pseudomonas aeruginosa, Pseudomonas sp. and Stenotrophomonas* sp. The microalgal strain was identified to be *Chlorella* sp. Further details on the isolation and identification would be sourced from Ismail et al. (2016).

2.2. Toxicity assays

Two different assays employing different microorganisms were used in the present study to assess the toxicity of the tested analgesics and some of their relevant metabolites (*p*-aminophenol). The microalgal toxicity assay was conducted according to Essam et al. (2014) using the isolated microalgal strain *Chlorella* sp. (A_1). The brine shrimp lethality assay (BSLA) was performed according to the protocol described in Ismail et al. (2016), this assay was used also to assess the detoxification efficiency of the treatment process. The toxicity of ketoprofen was determined in a previous study (Ismail et al., 2016) using the same mentioned organisms.

2.3. Tested analgesics

Paracetamol, aspirin and ketoprofen were kindly provided by El-Nile Company for Pharmaceutical Industries (Al-Amiriya, Egypt), Chemical Industry Development (CID) Company (Giza, Egypt) and Al-Amriya Pharmaceutical Industries Company (Alexandria, Egypt), respectively. The used concentrations of these analgesics to operate the PBR were adjusted to be sub-lethal concentrations, determined from the microalgal toxicity assay.

All tested Analgesics were analysed via HPLC using shimadzu LC-10 ADVP liquid chromatograph. The stationary phase and flow rate for all tested analgesics was Agilent Eclipse XDB-C18 4.6×250, 5 μm HPLC column and 1.25 $ml.min^{-1}$, respectively. Paracetamol and its main metabolite (*p*-aminophenol) were eluted with a mobile phase composed of 15: 85 of acetonitrile: 20 mM sodium phosphate buffer and detected at 245 nm. Aspirin and its main metabolite (salicylic acid) were eluted with a mobile phase composed of 50: 50 of water: methanol at pH of 2.9 adjusted by *ortho*-phosphoric acid, and detected at 283 nm. Ketoprofen was analysed using HPLC following the conditions described by Ismail et al. (2016).

2.4. Batch degradation of paracetamol and aspirin using the bacterial consortium (K_2) solely

Batch degradation experiments of ketoprofen under dark and illuminated conditions were performed in a previous study (Ismail et al. 2016). Similarly, the batch degradation experiments of 0.5 mM of aspirin or paracetamol were performed following the same protocol. A volume of 5 ml samples was periodically withdrawn every 4 and 12 h in case of aspirin and paracetamol, respectively. The concentrations of the drugs and main metabolites were determined by HPLC analysis.

2.5. Establishment and operation of the stirred-tank photobioreactor (STPBR)

The PBR was established using five liters glass tank continuously stirred by a magnetic stirrer at 200 rpm, illuminated with four LED lamps (illumination intensity of 5000 lx measured at the center of the reactor) and operated at room temperature (30 ± 2 °C).

Initially, the PBR was filled with mineral salt medium (MSM) supplemented with 2000 $mg.l^{-1}$ $NaHCO_3$ and inoculated with 10% v/v of A_1K_2 consortium. The microalgal culture was initially adjusted to contain 10 $mg.l^{-1}$ of chlorophyll-a while K_2 consortium suspension was initially adjusted to have an absorbance of 0.125 at 600 nm. The composition of MSM (per liter) is: (pH 7.0 ± 0.2) 4 g KH_2PO_4, 4 g K_2HPO_4, 2 g $(NH_4)_2SO_4$, 500 mg $MgSO_4{\cdot}7H_2O$, 0.01 mg $CaCl_2{\cdot}2H_2O$, 0.01 mg $FeSO_4{\cdot}7H_2O$, 1 ml of trace elements stock solution-1 (0.55 $g.l^{-1}$ $MnCl_2{\cdot}4H_2O$, 0.068 $g.l^{-1}$ $ZnCl_2$, 0.12 $g.l^{-1}$ $CoCl_2{\cdot}6H_2O$, 0.12 $ml.l^{-1}$ $NiCl_2{\cdot}6H_2O$ and 0.085 $ml.l^{-1}$ $CuCl_2{\cdot}2H_2O$) and 100 μl of trace elements stock solution-2 (3.1 $mg.l^{-1}$ H_3BO_3, 12 $mg.l^{-1}$ $Na_2MoO_2{\cdot}2H_2O$, 13 $mg.l^{-1}$ $Na_2SeO_3{\cdot}5H_2O$ and 16.5 $ml.l^{-1}$ $NaWO_4{\cdot}2H_2O$).

Once microbial growth was established as indicated by observing a significant increase in the optical density (at least three times increase), the artificial wastewater was added to the PBR in a fed batch mode. The tested artificial wastewaters were composed of MSM and supplemented with sub-lethal doses of the analgesics mixture ranging from 0.25/0.5/0.5 to 0.5/0.5/0.5 mM of Paracetamol/Aspirin/Ketoprofen (P/A/K), respectively. Initially, the PBR was operated at a relatively short HRT of 3 days. The PBR was

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