Production of PHAs with enhanced properties from sugar-based wastewater in a two stage process

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Abstract
The experimental design of the study was based on two different microbial processes, i.e. anaerobic fermentation of a sugar-based wastewater via mixed acidogenic cultures and aerobic polymerization of the produced acids and alcohols for poly-hydroxy-alkanoates (PHAs) production, via enriched cultures. For the first stage of the combined process, a continuous up-flow column reactor (UFCR) and a continuous stirred tank reactor (CSTR) were developed. For the optimization of the performance of both reactors, the effect of main parameters influencing the distribution of the produced acids was investigated. The liquid effluents of the reactors, containing fatty acids and ethanol were forwarded to the second stage of the combined process. A sequential batch reactor (SBR) was used for the second, aerobic stage of PHAs production
Keywords: PHAs, acids, sugar-based wastewater

1. Introduction

Plastics derived from petroleum may have provided very light, strong and economical materials for daily use for over 50 years but their extensive use and challenging disposal, have caused a cumulative environmental burden. Hence, there is an imperative need for the production of *biodegradable* and at the same time durable, polymers from renewable resources, which could sufficiently replace plastics in everyday uses, minimizing thus the environment impact. There are different approaches for the production of different types of *bioplastics,* which include the employment of chemical processes only, such as in the case of polycaprolactone (PCL), combination of biological and thermo-chemical processes such as in the case of polylactide (PLA) or entirely microbial processes such as in the case of polyhydroxyalkanoates *(PHAs)*, with the latter attracting increasing interest nowadays due to their unique properties.

PHAs constitute a family of aliphatic polyesters that can be accumulated intracellularly, serving as carbon reserves, by more than 300 microorganisms under unbalanced growth conditions. Sustainable production of such *microbial bioplastics* could be achieved through the bioconversion of various substrates, via pure (PMC) or mixed microbial cultures (MMC). The most important hurdle however to the commercialization of such bioprocesses is the cost of the chosen carbon source, which amounts up to 50% of the total production cost. Indeed, current industrial processes for PHA production utilize high value carbon sources, including sucrose, glucose and vegetable oils, either in a purified form or as hydrolysates (or extracts) from agricultural crops. In order to minimize the total production cost research initiatives should focus on the development of technologies that utilize cost efficient raw materials, such as waste streams and residual biomass of negligible cost (or even of high disposal cost) for the production of biopolymers. On one hand, such a practice would dramatically low the cost of bioplastics production, and on the other, it would also derive value from a hetero non-profitable process, i.e. waste treatment.

2. Overall Approach

2.1. Motivation

In line with the National and European strategic objective for a circular zero-waste economy, the present study aims to develop *environmentally friendly and economically viable bio-conversion processes for the bioconversion of sugar-based wastewater to PHAS*. According to the waste management hierarchy (Figure 1), the successful *reuse* of the wastes as raw materials for the production of new products is the most desirable option after prevention or minimization. Consequently, their bioconversion via biochemical and biological conversion processes, i.e. mild and environmentally friendly processes, are the most appropriate option for their prioritization at the top of the pyramid. The ultimate goal is the generation of PHAs based materials with adequate properties to be used for *food packaging* applications. As such, the utilization of those wastes streams towards PHAs is expected to eliminate severe environmental and economic burdens caused by the current ways of disposal, treatment, or recycling of food waste streams into a *circular economy* motto.

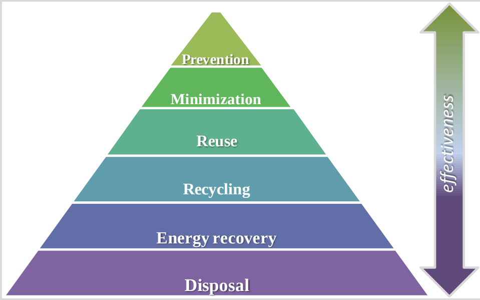

Figure 1: Hierarchy of waste management options

Among them focus is paid on developing methodologies for the recovery and/or generation of different types of carbon sources which could act as different precursors for the production of copolymers and blends with enhanced properties.

2.1. Methodology for the production of PHAs

In the frame of the study various strategies were developed for the efficient bioconversion of out-of-date fruit and vegetable juices and juice processing wastes i.e carbohydrate based substrates to PHAs and also for the production of tailor-made products with constant qualities. Those are based on the 1. development of different feeding and reactors operational modes using both PMC and MMC, 2. the modification and improvement of the final products.

As such, *a two stage process*, which has already been tested on other types of wastewaters and wastes (Ntaikou et al., 2009; Ntaikou et al., 2018) was applied based on two different microbial processes, i.e. anaerobic fermentation wastewater via MMC and aerobic polymerization of the produced short chain fatty acids for PHAs production, via enriched MMC (Figure 2). For the first stage of the combined process, two different types of continuous acidogenic systems are used i.e. a conventional CSTR type reactor and a continuous up-flow column reactor (UFCR). In the UFCR, microbial cells were immobilized onto ceramic beads, so as to prevent washout in high hydraulic retention times (HRTs). For the optimization of the performance of both reactors the following parameters were selected: initial concentration of sugars, pH and HRT.

The liquid effluent of both reactor, containing mainly fatty acids and residual sugars were forwarded to the second stage of the combined process. A sequential batch reactor (SBR) was used for the second, aerobic stage of PHAs production. The inoculum used for the start up of SBR was an enriched culture that was developed either by thermally pretreated soil, or activated sludge, subjected to nutrient limitation.

Emphasis is then given on the optimal recovery of the intracellular PHAs and also on their detailed characterization in terms of their composition, structure and molecular masses, their thermal and mechanical properties, as well as their biodegradability.

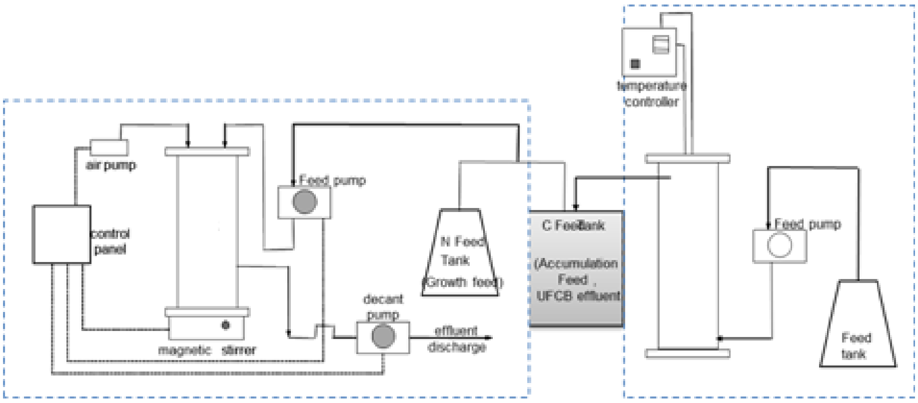

Figure 2. Two stage process for the bioconversion of sugar-based wastewater to PHAs, based on MMC.

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