



# Lipid-enhancement of activated sludges obtained from conventional activated sludge and oxidation ditch processes



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## HIGHLIGHTS

- This study demonstrated lipid-enhancement of activated sludges via fermentation.
- Significant increase in the lipid content of the sludges was observed.
- Triacylglycerides comprised more than 50% of total lipids from enhanced sludge.
- Regardless of the source, the resulting sludges have similar fatty acid profile.

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## ABSTRACT

Lipid-enhancement of activated sludges was conducted to increase the amount of saponifiable lipids in the sludges. The sludges were obtained from a conventional activated sludge (CAS) and an oxidation ditch process (ODP). Results showed 59–222% and 150–250% increase in saponifiable lipid content of the sludges from CAS and ODP, respectively. The fatty acid methyl ester (FAMES) obtained from triacylglycerides was 57–67% (of total FAMES) for enhanced CAS and 55–73% for enhanced ODP, a very significant improvement from 6% to 10% (CAS) and 4% to 8% (ODP). Regardless of the source, the enhancement resulted in sludges with similar fatty acid profile indicating homogenization of the lipids in the sludges. This study provides a potential strategy to utilize existing wastewater treatment facilities as source of significant amount of lipids for biofuel applications.

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## 1. Introduction

Wastewater treatment operations involve biological processes, which can be separated broadly into two categories: fixed film and suspended growth systems. Fixed film systems include trickling filters and rotating biological contactors while suspended growth systems include various modifications of the activated sludge process (Spellman, 1999). All biological processes produce solids (also referred to as “biosolids” or sludge) that need to be treated prior to disposal. Due to ecological, economic, social and legal factors, sludge treatment and disposal is considered to be the bottleneck of most wastewater treatment plants (WWTPs) (Pérez-Elvira et al., 2006). Thus, sludge minimization and more importantly, its utilization for production of high-value products could greatly benefit WWTPs.

The utilization of waste sludge as source of feedstock for biofuel production has been the subject of several studies (Dufreche et al., 2007; Mondala et al., 2009; Revellame et al., 2010; Siddiquee and Rohani, 2011). These studies have focused on biodiesel production from the saponifiable lipids present in activated sludge. Due to relatively low concentration of such lipids, activated sludge is not economically competitive at current prices of petroleum-based fuels. Recent study showed that raw activated sludge contains significant amount of unsaponifiable compounds (i.e. hydrocarbons, and sterols) in addition to saponifiable lipids [i.e. free fatty acids (FFAs), monoacylglycerides (MAGs), diacylglycerides (DAGs), triacylglycerides (TAGs), phospholipids (PLs), wax esters (WEs), steryl esters (SEs) and polyhydroxyalkanoates (PHAs)] (Revellame et al., 2012). Unsaponifiable lipids could be important precursors for a variety of applications/products, but are unwanted in biodiesel production technology.

The viable utilization of wastewater sludge for biodiesel production is constrained by several factors:

- (1) Low saponifiable lipid yield.
- (2) Differences in the types of wastewater being treated (i.e. domestic, food, agricultural, clinical and industrial).

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## Nomenclature

CAS	Conventional activated sludge	PHAs	Polyhydroxyalkanoates
DAGs	Diacylglycerides	PLs	Phospholipids
FAMEs	Fatty acid methyl esters	SEs	Steryl esters
FFAs	Free fatty acids	TAGs	Triacylglycerides
HB	Hydroxybutyric acid	WEs	Wax esters
HV	Hydroxyvaleric acid	WWTP	Wastewater treatment plant
MAGs	Monoacylglycerides		
ODP	Oxidation ditch process		

- (3) Differences in configurations of existing WWTPs (i.e. conventional activated sludge, oxidation ditch, trickling filter, rotating biological contactors).
- (4) Differences in microbial populations involved in biological treatment.

These factors could result in huge differences in yields and speciation of lipidic materials present in wastewater sludge. In turn, this would require specific fuel conversion strategy for each particular factor combination. This scenario is not desirable especially for low capacity WWTPs.

To address these factors, researchers have looked at several strategies. These include utilization of pre-treated wastewater sludge as substrate for yeast-based biodiesel (Seo et al., 2013), and addition of oil-accumulating microbial consortium to the indigenous microorganisms in the wastewater (Hall et al., 2011). In a recent study, Mondala et al. (2012) proposed a modification of existing WWTPs that can possibly address these issues. The proposed concept involved an additional lipid-accumulation unit where the waste activated sludge from a WWTP, is subjected to an environmental condition (stressed condition) that facilitates lipid production. Results of their batch fermentation experiments using glucose and ammonium sulfate as carbon and nitrogen sources, respectively, showed a maximum lipid yield of  $17.5 \pm 3.9\%$  (cell dry weight) can be obtained at a glucose loading of 60 g/L with a corresponding carbon–nitrogen mass ratio of 70:1. At this fermentation condition, they obtained a biodiesel yield of  $10.2 \pm 2.0\%$  (cell dry weight) (Mondala et al., 2012). However, they applied the enhancement on sludge from a conventional sludge process alone. For sustainable utilization of WWTPs as source of lipid feedstock, the enhancement needs to be applied on sludges from other treatment configuration.

It is well documented that bacteria can synthesize lipid storage compounds (i.e. acylglycerides) under stressful conditions (i.e. nitrogen, oxygen and nutrient limitation) provided that there is an excessive supply of carbon source (Alvarez, 2006; Alvarez et al., 1997; Alvarez and Steinbüchel, 2002). Commonly, nitrogen limitation is the physiological stress that is being used to channel metabolic fluxes to lipid accumulation (Courchesne et al., 2009; Ratledge and Wynn, 2002). Studies on the biodiesel production from activated sludge taken from a municipal WWTP indicated that a yield of around 3–6% (dry weight) could be obtained from this feedstock (Dufreche et al., 2007; Mondala et al., 2009; Revellame et al., 2010). Based on the economic analysis conducted by Revellame et al. (2011), a yield of more than 10% (sludge dry weight) is necessary for this feedstock to be economically viable (Revellame et al., 2011). Lipid-enhancement, as depicted in Fig. 1, can be one strategy to achieve the required biodiesel yield; wherein a portion of the wastewater input to the treatment plant is used as carbon and nutrient source. However, to induce lipid-accumulation, additional carbon and nutrient sources might be needed. This could affect the economics of this feedstock negatively, but might

be compensated by using relatively inexpensive carbon sources (i.e. lignocellulosic materials). In the United States alone, approximately 1.3 billion tons per year of lignocellulosic biomass could be used sustainably for biofuel production (Kosa and Ragauskas, 2011).

In this study, lipid-enhancements were applied on activated sludges from conventional activated sludge (CAS) and oxidation ditch process (ODP) configurations. Activated sludge process modifications include CAS, step aeration, completely mix, pure oxygen, contact stabilization, extended aeration and ODP (Spellman, 1999). Among these modifications, CAS and ODP were chosen since they are the most commonly used ones in the United States. According to US EPA, 23% of all WWTPs employing the activated sludge process (~1690) utilize CAS. On the other hand, ODP comprises 40% (including extended aeration systems) (US EPA, 2010). ODP is fundamentally similar to extended aeration system (Arceivala and Asolekar, 2007).

CAS and ODP were also chosen based on their operational requirement; CAS requires primary treatment (clarifier) while ODP does not. The main purpose of primary treatment in a WWTP is for removal of settleable and floatable solids. It is also in this section where oil and grease are skimmed along with other floatable materials (Spellman, 2009). The amount of lipids (oil, grease, fats and fatty acids) in most municipal wastewater sums up to about 30–40% of its total chemical oxygen demand. Studies on their fate in biological waste treatment indicated that in addition to biodegradation, they are also adsorbed by the biomass (Chipasa and Mędrzycka, 2006). Thus, the absence of primary treatment could have a significant impact on the amount and speciation of lipids present in the sludges after lipid-enhancement.

This study was conducted to address some of the factors mentioned earlier that prevent viable utilization of wastewater sludge for biofuel production. Particularly, this work seeks to increase the amount of saponifiable lipids in sludges from two WWTP configurations: CAS and ODP. Furthermore, this study aims to determine the effect of enhancement on the speciation of lipids in the sludges. For all the experiments, glucose and ammonium sulfate were used as carbon and nitrogen sources, respectively. This study provides initial and to date the only report on solidifying the concept of WWTPs as source of feedstock for biofuel applications.

## 2. Experimental section

### 2.1. Activated sludge collection and preparation

Three batches of activated sludges were collected from WWTP in Tuscaloosa, AL, USA. (Hilliard Fletcher municipal WWTP) and Tupelo, MS, USA. They were collected in the months of October, November and February (coded O, N and F, respectively) during the plants' normal operations. The Tuscaloosa plant utilizes CAS treatment configuration, while the Tupelo plant utilizes ODP. Samples were collected in 1-L plastic containers from the return

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