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# Effect of citric acid induced deflocculation on the ultrasonic pretreatment efficiency of dairy waste activated sludge

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ABSTRACT

In this investigation, the application of citric acid was explored for the removal of extracellular polymeric substance (EPS) from waste activated sludge (WAS), followed by ultrasonic pretreatment, which enhanced the subsequent anaerobic biodegradability. EPS was removed with 0.05 g/g SS of citric acid. The chemical oxygen demand (COD) solubilization and suspended solids (SS) reduction that occurred for specific energy input of 171.9 kJ/kg TS, in deflocculated (EPS removed and ultrasonically pretreated) sludges were found to be 22.70% and 20.28% and was comparatively higher, than the flocculated (with EPS and ultrasonically pretreated). The biogas yield potential of flocculated and deflocculated sludges (specific energy input – 171.9 kJ/kg TS) was found to be 0.212 L/(g VS) and 0.435 L/(g VS), respectively. Accordingly, the deflocculation and ultrasonic pretreatment improved the anaerobic biodegradability efficiently. Thus, this chemo mediated sonic pretreatment is an effective method for enhancing biodegradability and improving clean energy generation from WAS.



## 1. Introduction

Dairy industry is one of the industries producing waste water rich in organic matter and waste materials which leads to bad odour. Even though water management in the dairy industry is well documented, effluent production and disposal remains problematic for the dairy industry. Sludge treatment and disposal in wastewater treatment plants are challenging, requiring a significant financial outlay for both capital and operational costs [1]. Proper treatment and disposal of excess sludge is quite expensive and can account for up to 60% of the total operating cost of a wastewater treatment plant [2]. This sludge must undergo some treatment in order to reduce its associated volumes, to improve its character and to reduce the associated health problems and hindrance. This treatment will (i) first reduce the water content of the raw sludge, (ii) transform the highly putrescible organic matter into a relatively stable or inert organic and inorganic residue, and (iii) finally, condition the residue to meet disposal acceptance regulation [3]. For these reasons, anaerobic sludge digestion optimizes Waste water treatment plants (WWTP) costs and is considered a major and essential part of a modern WWTP [4]. During anaerobic digestion of sludge, the enzymatic hydrolysis of solids is reported to be the rate-limiting step due to the complex structure of waste activated sludge (WAS). In order to improve the rate of hydrolysis and the anaerobic digestion performance, sludge disintegration was developed as a pretreatment process to accelerate the anaerobic digestion and to increase the degree of stabilization [5]. Various pre-treatments, including mechanical, thermal, physical, chemical and biological interventions, have been studied recently. All pretreatments result in a lysis or disintegration of sludge cells, thus releasing the solubilized intracellular materials into the liquid phase and transforming refractory organic materials into biodegradable species, therefore making more materials readily available for microorganisms [6]. It is shown that these pretreatments enhance the biogas generation. Among these alternate methods, physical treatment of sludge plays an important role since it favors solubilization of particulate matters in liquid phase. Once the organics are solubilized, the sludge becomes more convenient for further treatment. The physical treatment methods include mainly ultrasonication and ozonation. Among these two methods, ultrasonication is a well-known physical method for cell disruption. The ultrasonication is an emerging and promising mechanical disruption technique for sludge disintegration due to several inherent merits like efficient sludge disintegration (>95%), improvement in biodegradability, improved biosolids quality, increase in methane percentage in biogas, no chemical addition,

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and less retention time [7]. Sonication is also a novel approach with high application potential in sludge disintegration for its effectiveness and simple operation [8]. In this study, extracellular polymeric substances (EPS) are removed from the sludge to further increase the efficiency of ultrasonic pretreatment. EPS have been reported to aid typically in the formation of a gel-like network that keeps the bacteria together in biofilms, which causes the adherence of the biofilms to surfaces and protects the bacteria against noxious environmental conditions [9]. EPS composed of a variety of organic substances, such as carbohydrates, proteins, nucleic acids, and lipids, plays an imperative role in bioflocculation [10]. A majority of protein in EPS is bridged by divalent ions like $Ca^{2+}$ and $Mg^{2+}$. Cation-binding agents disturb the sludge flocs structure by removing bridging ions (e.g. $Ca^{2+}$, $Mg^{2+}$, $Fe^{2+}$ and $Mg^{3+}$). The released organic matter could be potentially used in anaerobic degradation [11]. Therefore, it is mandatory to remove the EPS before pretreatment in order to reduce the organic solid contents. The prime objectives of this study are to (1) remove EPS with a suitable cation binding agent, citric acid, (2) to evaluate the effect of EPS removal in subsequent ultrasonic pretreatment, (3) to analyze the kinetic parameters of pretreatment and (4) to investigate the anaerobic biodegradability of deflocculated (EPS removed and ultrasonically pretreated) WAS.

## 2. Materials and methods

### 2.1. Sludge sampling and characterization

Dairy waste activated sludge was sampled from Aavin dairy effluent treatment plant at Madurai. Samples were collected and stored at 4 °C. The characteristics of the raw sludge were as follows: total COD (TCOD) was 10,000 mg/L, soluble COD (SCOD) was 400 mg/L, pH was 6.91, total solids (TS) concentration was 12,560 mg/L, suspended solids (SS) concentration was 7000 mg/L, volatile solids concentration was 5600 mg/L, total alkalinity concentration was 985 mg/L, protein concentration was 72.6 mg/L, carbohydrate concentration was 6.1 mg/L and DNA concentration was 3.2 mg/L.

### 2.2. Optimization of citric acid dosage for removal of EPS

Citric acid dosage optimization was performed in nine 250-mL conical flasks containing 100 mL of sludge and citric acid in the range of 0.01–0.5 g/g SS. The contents of the conical flasks were mixed continuously using an orbital shaker for 1 h at 150 rpm. The experiments were carried out at a room temperature of 30 °C. After incubation, the samples were centrifuged at 10,000 rpm for 15 min to obtain the soluble EPS in the supernatant.

### 2.3. Ultrasonic pretreatment

Ultrasonic pretreatment was carried out using a Sonopuls ultrasonic homogenizer (Bandelin Sonopuls HD 2200, Berlin, Germany). This apparatus was equipped with a VS 70 T probe with an operating frequency of 20 kHz and a supplied power of 200 W. For each experiment, 500 mL of sludge were placed in a glass beaker without temperature adjustment (no cooling) and an ultrasonic probe was submerged in the sludge to a depth of 2 cm above the bottom of the beaker. The effect of this pretreatment, which mainly depends on the treatment time, was evaluated by taking samples at different times (10 s, 20 s, 30 s, 40 s, 50 s, 1 min, 2 min, 3 min, 5 min, 7 min, 10 min, 20 min, 30 min) to study the effect of sludge disintegration. Specific energy was considered as a main variable parameter for evaluation of disintegration performance of the sludge [12,13]. It is determined by using ultrasonic power ($P$), ultrasonic time ($t$), sample volume ($V$) and initial total solid concentration ($TS_0$) according to the following Eq. (1):

$$SE\ (kJ/kg\ TS) = P\ (W) \times t\ (s)/V\ (L) \times TS_0(g\ L^{-1}) \quad (1)$$

### 2.4. Biochemical methane potential (BMP) assay

A biochemical methane potential (BMP) assay was performed to evaluate the biogas recovery from sludge after ultrasonic pretreatment. The biodegradability assays were conducted in anaerobic batch reactors of 300 mL capacity serum bottles with hermetically sealed stoppers, and an experiment was carried out in 4 serum bottles A–D respectively. Each serum bottle was filled with 150 mL of inoculum and 50 mL of substrate. A blank treatment with150 mL of inoculum was mixed 50 mL of flocculated (with EPS and ultrasonically pretreated alone) sludge. The inoculums needed to be quite active, with a good adaptability and a low endogenous respiration [14]. For these experiments, rumen bacteria from the digestive tract of a cow were used as an inoculum. The rumen fluid seeded in a bio digester has a significant effect on the cumulative biogas production and biogas production rate [10]. The initial pH was recorded as neutral before the start of BMP assay tests. After adding the substrates and inoculum, the reactors were closed with a rubber septum and an aluminum seal to make them air tight and was subsequently purged with nitrogen gas at the rate of 10 mL/s for 20 min into the reactors in order to maintain anaerobic conditions. An internal temperature of 35 °C was maintained by incubating the reactors in a temperature controlled mechanical shaker (220 rpm). The biogas was measured by inserting a needle into the septum. Gas accumulation produced during the incubation was collected using a syringe. Batch reactors were operated with a residence time of 40 days. The modified Gompertz equation was used to study the cumulative biogas generation and the kinetics of biogas production:

$$B_t = B * \exp[-\exp[R_b/B * \exp(\lambda - t) + 1]] \quad (2)$$

where $B_t$ is the cumulative biogas produced (mL) at any time ($t$), $B$ is the biogas production potential (L/(g VS)), $R_b$ is the maximum biogas production rate (L/(g VS d)), and $\lambda$ is the lag phase (days), which is the minimum time taken to produce biogas, or the time taken for bacteria to acclimatize to the new environment. The constants $B$, $R_b$, and $\lambda$ were determined using the non-linear regression method with the help of Polymath software.

### 2.5. Analytical methods

The concentrations of total solids (TS), suspended solids (SS), chemical oxygen demand (COD), alkalinity and turbidity were measured according to standard methods [15]. EPS proteins, EPS carbohydrates, and DNA were determined photocolorimetrically using an ultraviolet–visible spectrophotometer. The amount of proteins in the extracted EPS was determined by Lowry's method [16] using bovine serum albumin as the standard compound and measuring absorbance at 620 nm. The carbohydrate was quantified by anthrone–sulfuric acid method with glucose as the respective standard [17] measuring absorbance at a wavelength of625 nm. The DNA was quantified by Diphenylamine colorimetric method with *Escherichia coli* DNA as the respective standard [18]. The enzyme activity (Protease and amylase) was analyzed spectrophotometrically according to the methods followed by [19]. All the experiments were done in triplicate and the standard errors were calculated with respect to statistical analysis. The total amount of extracted EPS was measured by the sum of protein and polysaccharides. The COD solubilization (%) was calculated by using the Eq. (3):