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Protein Recovery from Dairy Sludge by Fenton Process

Naser Mehrdadi[1], Gholamreza Nabi Bidhendi[1], Saeid Nazemi[2*], Aliakbar Roudbari[3]

[1] Dept. of Water and Wastewater Engineering, School of Environment, University of Tehran, Tehran, Iran.
[2] Dept. of Environment, Aras International Campus, University of Tehran, Tehran, Iran.
[3] Center for Health Related Social and Behavioral Sciences Research, Shahroud University of Medical Sciences, Shahroud, Iran.

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Abstract

Background: *Holding excessive amount of sludge has turned into a major problem for the wastewater treatment plants. Nucleic acids, enzymes, proteins, and polysaccharides are some organic materials which could be found in the sludge. The main objective of this study was to investigate the efficiency of Fenton process in protein recovery from dairy sludge.*

Methods: *Our case forthestudy was the waste activated sludge produced at the wastewater treatment plant of Fajr dairy industry in Shahrood, Iran. Fenton process was applied to a 1.5 L sludge sample. At first, the pH of the sludge was adjustedto 3 using H_2SO_4. The second stepwas the addition of Fe(II) at certain concentrations.Then, different H_2O_2 concentrations were added to the sample. The mixed sample was stirred at 120 rpm for 6 h and was neutralized with $Ca(OH)_2$. The sludge was dewatered in the pilot-scale filter pressandfiltered. The solubleprotein content in the supernatant of the disintegrated sludge derivedfrom the Fenton process was recovered by dialysis and dried at −40°C for 24 h.*

Results: *The results showed that after the Fenton process, SSi, TCODi, SCODi, and SCODa levels were 11275,13800,115, and 3450 mg/Lrespectively. Also, after the Fentonprocess,theconcentration of the soluble proteins increased from 52.48 to 1732 mg/L,whereas after subsequent protein recovery, its concentration in the supernatant was 1180 mg/L.*

Conclusions: *Based on the findings, theproteinrecoveredfrom the excess sludgethroughout theFenton process can be used as animal feed.*

Keywords: Protein, Sludge, Dairy industry.
***Corresponding to:** S Nazemi, **Email:** nazemi@shmu.ac.ir
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Introduction

Dairy industry is one of the prime industrial sectors in Iran with an annual growth rate of more than 20%. The industry produces a large amount of wastewater with high BOD level. Treating of dairy wastewater with activated sludge process generates a large amount of sludge which needs to be managed.[1]

The management of excess sludge is difficult for the wastewater treatment plant operators because sludge disposal accounts for up to 60% of the total operating costs of the treatment plant.[2]

Various methods like ultrasonic treatment,[3] ozone oxidation,[4] alkaline treatments,[5]thermal treatments,[6] Fenton process,[7]and biological hydrolysis with enzymes[8] were investigated for sludge disintegration by several researchers in lab and on a pilot-scale. Often, these methods are costly and produce harmful by-products also, they have little effect on reducing the amount of sludge. The excess sludge treatment cost has reached a high level due to land shortage and environmental conservation. Thus, developing new technologies is vital for the disposal and reuse of the excess sludge.[1]

The advantage of directly extracting useful biomaterials from the excess sludge and utilization of physicochemical or biological process in order to turn it into a material with new interesting characteristics has been widely developed. Nucleic acids, enzymes, proteins, and polysaccharides are some organic materials which could be found in the sludge.[9]

Proteins account for about 50% of the dry weight of bacterial cells.

It is a vital constituent of animal diet. Thus, researchers are looking for alternative sources of protein. The existing cellular proteins in the mixed microbial cultures of activated sludge from sewage treatment plants have been considered as the only suitable substitution, full of proteins, for our previous resources which can compensate their protein shortages.[10]

For protein recovery, solubilization of intracellular materials in excess sludge must be achieved first. Many biological, physical, or chemical treatments, such as alkali treatment[11] and ultra-sonication,[12] have been reportedly used to disrupt the sludge structure.

It is easy to notice that after the disintegration, despite an increase in the concentrations of soluble proteins and soluble chemical oxygen demand (SCOD), a decrease in the size of the floc and the amount of suspended solids (SS) would occur.[13-15]

The methods to recover proteins from sludge have been studied for many years. Previous investigations have proved that directly using the obtained protein as a food resource for animals would lead to negative results, and an increase of diseases among animals would be inevitable.[16]

So, efforts have been made to recycle the proteins from sludge. One of these methods is the Fenton process, which is one of the commonly used advanced oxidation processes. Fenton reagent is a mixture of ferrous iron and H_2O_2. The ferrous iron initiates and catalyzes the decomposition of H_2O_2 resulting in the generation of highly reactive hydroxyl (OH^o) radicals.[17]

The OH^o radical is the main reactant in the process capable of decomposing a number of organic substances via oxidation. The rate and extent of the Fenton reactions are dependent on

the system parameters including iron and H_2O_2concentrations and the solution pH. The objective of this study was to investigate the efficiency of Fenton process in protein recovery from dairy sludge.

Materials and Methods

The waste activated sludge was obtained from a wastewater treatment plant at Fajrdairy Industry, Iran. The samples were collected and stored at 4℃. Table 1 shows the characteristics of the raw sludge.

Table 1. Properties of raw sludge

Parameters	Raw Sludge
pH	7.2±0.18
Suspended Solids (SS, mg/L)	11275±580
Volatile Suspended Solids (VSS, mg/L)	21450±1920
Soluble Chemical Oxygen Demand (SCOD, mg/L)	285±23
Total Suspended Solids (TSS, mg/L)	27500±1830
Total COD (TCOD, mg/L)	7300±456
Soluble protein (mg/L)	132±11
Soluble Carbohydrate (mg/L)	7.5±0.03

The Fenton process was applied to a 1.5 L sludge sample. At first, the pH of the sludge was adjusted to 3 using H_2SO_4. The second step was the addition of Fe(II) at certain concentrations. Then, different H_2O_2 concentrations were added to the sample. The mixed sample was stirred at120 rpm for 6 h and neutralized with $Ca(OH)_2$. In the Fenton experiments, analytical grade $FeSO_4{\cdot}7H_2O$ was used as the source of Fe(II) and purchased from Merck. H_2O_2solution (37% [w/w]) in a stable form, H_2SO_4 (98%˚99%), and NaOH were all provided by Merck.

In order to evaluate the disintegration efficiency of the Fenton method, the TCOD, SCOD, and soluble protein levels were measured after the sludge disintegrated. The following standard methods were used to measure the SS and VSS.[18]

For increasing the accuracy of the measurement, we repeated every experiment three times.

The supernatant obtained through the centrifuging process of the disintegrated sludge at 7000rpm for about 30min at a temperature of 4˚C was used to determine the SCOD and soluble protein levels. The concentrations of the COD and proteins, respectively, were measured by the HACH 5000 method using a HACH DC/2500 spectrophotometer and by the Lowry method,[19] using bovine serum albumin as a reference protein. The increase in SCOD was calculated by the below formula:

$$\text{COD solubilization rate}(\%) = \frac{\text{SCODa} - \text{SCODi}}{\text{TCODi\_SCODi}} \times 100$$

Where SCODi and SCODa are the soluble COD before and after the treatment, respectively, and TCODi is the initial TCOD.

The sludge was dewatered in the pilot-scale filter press and then filtered. The soluble protein in the supernatant of the disintegrated sludge was recovered by dialysis and dried at 40 ℃ for 24 h. The freeze-dried process was sealed to prevent the reabsorption of moisture and protect against spoilage for many years.

The recovered powder is composed of the moisture, crude protein, crude fat, crude fiber, and ash which were measured by following the Iranian National Standardization Organization method of feed analysis.[20] After decomposing the powder according to the aqua Regia method, and then filtering it using a filter paper, the concentrations of heavy metals could be determined (GF Whatman).An atomic absorption spectrometer was used to determine the concentrations of heavy metals in the filtrate (Varian, USA). Additionally, aflatoxin B1and Salmonella D groups were measured by following the guidelines of the Institute of Standards and Industrial Research of Iran method of feed analysis.[20]

Results

After the Fenton treatment of the excess sludge at pH 3 for 6 h, the SSi, TCODi, SCODi, and SCODa readings were 11275, 13800,115, and3450 mg/L, respectively, which yielded a COD solubilization rate of 32%. After the Fenton treatment, the soluble protein concentration increased from 52.48to 1732 mg/L, whereas after the subsequent protein recovery, its concentration in the supernatant at pH 3.3 was 1108 mg/L. However, it was estimated that 40.5% of the released soluble proteins from the disintegrated sludge was produced at pH 3.0.

Table 2. Concentrations of heavy metals

Element Unit (mg/kg)	Original excess sludge	Recovered powder	Legal standard[c]
As	0.08	ND[a]	2
Ca	1850	1027	_b
Cd	0.01	ND	1
Cu	256	223.4	_
Fe	2235	2780	_
Hg	0.02	ND	0.4
Pb	21.35	7.1	10
Zn	456.4	168.2	-

a Not detected.
b Not prescribed in the regulation.
c The lowest value in the legal standard of various feeds

The components of the recovered powder which was used as animal feed were analyzed to determine their nutritional values. The main portion of the recovered powder consisted of crude protein (51.73%). The other materials recovered included nitrogen-free extract (NFE) (20.9%), crude ash (13.8%), crude fat (10.5%), moisture (3%), and crude fiber (0.07%).

The measured concentrations of heavy metals in the primary excess sludge and the recovered powder are listed in table 2. The primary sludge contains a variety of hazardous heavy metals. It has been mentioned that heavy metals in wastewater are concentrated into sewage sludge through the primary and secondary treatment processes.[21]For example, the heavy metal scan be absorbed by biomass and get precipitated by some anions, such as sulfide, and accumulate in the excess sludge.[22]During the recovery processes of proteins, significant amounts of the heavy metals in the raw sludge were removed. Furthermore, aflatoxinB1 and Salmonella D, indicators of the presence of pathogenic bacteria, were not observed (data not shown). As a result, heavy metals, harmful toxins, and microorganisms which are contained in the primary excess sludge can be removed in significant amounts through the processes of cell disintegration and protein dialyzation.

Discussion

The extent of the Fenton reactions is dependent on the system parameters including iron, solution pH, and H_2O_2 concentration.

Figure 1 shows that the concentration of VSS decreased with the increase of H_2O_2. Kitis et al. showed that the OH radical is the main reactant in the process capable of decomposing organic substances via oxidation.[17]

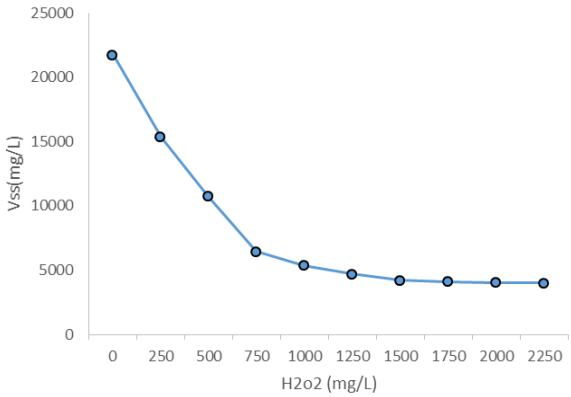

Figure 1. Impact of peroxide on the disintegration of excess sludge

Figure 2 shows that the concentration of soluble proteins increased simultaneously with the increase of H_2O_2 particularly in the concentration range of 1500˚ 2500 mg/L.

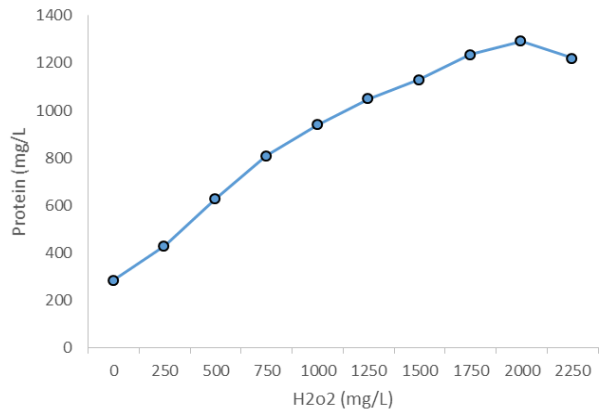

Figure 2. Impact of peroxide on the concentration of protein

Another study on sludge revealed that with the disintegration of the sludge, an increase in the concentrations of SCOD and soluble proteins as well as a decrease of the sludge volume and the amount of SS were observed, which is consistent with the results of the present study.[13]

It was observed that at pH3, the protein concentration and COD solubilization increased.

Takumuraet al. applied a similar advanced oxidation method for the disintegration of sludge, and a high amount of SCOD was achieved at the pH 3, which is consistent with the results of the present study.

When studying the effects of H_2O_2 on the ultrasonically assisted sludge disintegration, the results showed that no increase in SCOD was achieved by using ultrasound along with oxidizing agents alone.

The protein concentration increased with the increase of H_2O_2which is in disagreement with the results of the studies published earlier.[23]

Figure 3 shows that the combination of $H_2O_2$and Fe^{2+} led to a rapid increase in the protein concentration and COD solubilization. The highest protein generation was achieved at a Fe2+/H_2O_2 ratio of 1.The soluble protein released from the disintegrated sludge was precipitated at pH 3.3, which is in contrast to the findings of the studies published earlier.[24]

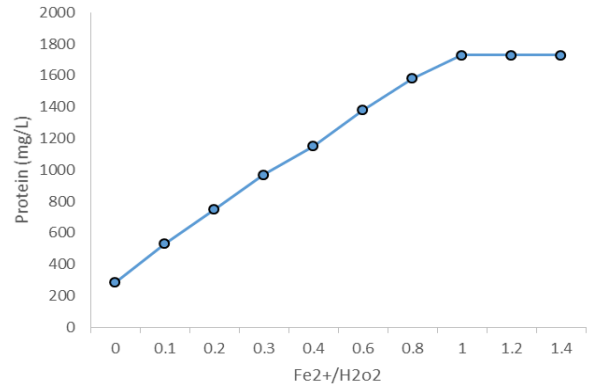

Figure 3. Impact of Fe2+ with peroxide on the concentration of protein

As shown in Figures 3 and4, with the increase inFe^{2+}/H_2O_2 ratio, VSS decreased as the protein concentration increased.

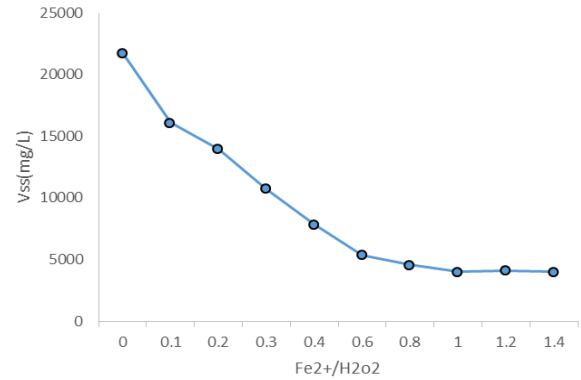

Figure 4. Impact of Fe2+ with peroxide on the disintegration of excess sludge

Due to the use of H_2O_2, there is an intensification of aerobic digestion, which further reduces VSS.

The main reason for the volatile suspended solids (VSS) reduction during the Fenton process was the rupture of the cell wall and release of extracellular and intracellular matter.[25]

Cellular proteins were obtained from the excess sludge of a dairy wastewater treatment plant. In comparison, the nutrient compositions of the recovered protein and those of dried Brewer s yeast and bone meal were alike. The heavy metal concentrations were lower than the standard quantity allowed.

The indicators of the presence of pathogenic bacteria, aflatoxin B1 and Salmonella D, were not detected. Finally, it

was shown that using the recovered protein from excess sludge as animal feed was feasible.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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