



PRE-TREATMENT OF DAIRY WASTE WATER USING BIOPOLYMERIC FLOCCULANT EXTRACTED FROM ORANGE PEEL

Shabna Banu A.M.¹, Meena John M.²

^{1,2}Department of Civil Engineering, Govt. Engineering College Trichur, (India)

ABSTRACT

Biopolymeric flocculants are attracting great research interests due to their biodegradable and nontoxic nature. Pectin is a biopolymeric flocculant, which can be extracted from fruit wastes, and thus the fruit waste can be reused. This study focuses on the suitability of pectin extracted from orange peel to treat dairy waste water. Pectin was extracted from orange peel. Extraction performances of two solvents (ie isopropyl alcohol and ethanol) were compared. Extraction from orange peel using isopropyl alcohol gave maximum pectin yield ie 39.01%. Detailed characterization of extracted pectin was carried out using FTIR analysis and SEM analysis. FTIR analysis confirms the presence of hydroxyl groups and polygalacturonic acid in the pectin molecules. The performance of pectin was evaluated by conducting coagulation-flocculation experiments using $FeCl_3$ as coagulant and extracted pectin as flocculant. Response surface methodology was carried out using Box Behnken design to find the optimum pH, coagulant dosage and flocculant dosage to maximize COD removal, phosphate removal, nitrate removal and turbidity removal. Optimum conditions were obtained as pH=4.00, coagulant dosage=99.49 mg/L and flocculant dosage=10.59 mg/L. At the optimum conditions, COD removal, phosphate removal, nitrate removal and turbidity removal were 48.32%, 69.46%, 62.29% and 99.91% respectively. Settling characteristics of sludge was evaluated based on sludge volume index. SVI index was obtained above 250 mg/L. Thus the sludge is having poor settling characteristics and high effluent turbidity.

Keywords: Box Behnken design, Coagulation-flocculation, Dairy waste water, Pectin, Response surface methodology

I. INTRODUCTION

Rapid industrialization contributes to large quantity of waste water and its treatment is highly imperative. Compared to other industrial sectors, the dairy industry uses a much greater amount of water for each ton of product and produce large amount of waste water. Due to the high pollution load of waste water, the milk processing industries discharging untreated or partially treated waste water causes serious environmental problems, thus appropriate treatments are required. Reducing the waste water treatment cost is gaining importance recently in view of environmental sustainability. Biological methods are usually used for the treatment of dairy waste water due to its biodegradable nature. But these methods consumes large amount of energy and also they are very complex. It necessitates the pretreatment of dairy waste water prior to biological



treatment. Coagulation- flocculation methods can be used as an effective pretreatment method for dairy waste water. Because these methods can remove COD, phosphate, nitrate etc from dairy waste water and thus reduces the organic loading of the waste water.

Chemically synthesized flocculants are playing dominant role in waste water treatment due to their low cost. But these flocculants have various limitations such as increased sludge production; non degradable nature and causes a lot of environmental problems. Biopolymeric flocculants are attracting great research interests due to their biodegradable and nontoxic nature. Since biopolymeric flocculants are free of secondary pollution, they could be a potential replacement for toxic chemical synthetic flocculants. Pectin is a biopolymeric flocculant, which can be extracted from different fruit wastes materials, and thus the fruit waste can be reused. It opens a new path for the management of solid waste and reduces the effects of sanitary landfills.

The increase in the demand for a proper solid waste disposal sites and the effects of sanitary landfills makes a problem in the management of solid waste. Food and organic waste from residential areas, commercial by-products and institutional uses produce the highest amount of waste at 63.1%, 76.8%, and 40.6%, respectively, as compared to mixed papers, mixed plastics, textiles, rubbers and leathers, yard wastes, glasses, ferrous, aluminum, and other wastes. It causes several environmental concerns such as leachate and run off, odor, vectors, and fires. Moreover, it is reported that only 1–2% of this waste is being recycled while the remainder is taken to disposal sites [1]. Hence, it is important to recycle the organic waste such as fruit waste into useful biopolymer flocculant.

This study investigates the effectiveness of orange peel to yield pectin using acid extraction method and compares two extraction solvent. It also studies the effectiveness of pectin for the pre-treatment of dairy waste water.

II. MATERIALS AND METHODS

2.1 Waste water sampling and preparation of synthetic waste water

The dairy waste water was collected from MILMA Dairy plant, Ramavarmapuram. The samples were tested for different waste water characteristics as per the standard methods (standard methods for the examination of water and waste water, 19th edition, 1995).

Synthetic waste water was used for the study. It was prepared based on the characteristics of collected dairy waste water. The quantities of constituents were found out by trial and error method.

2.2 Extraction of biopolymeric flocculant (pectin)

Acid extraction method was used. Orange was peeled and washed with distilled water in order to remove dirt, dust and the residues of the pesticide spray. The peels were cut into small pieces, then it was blanch with boiling water for 5 minutes to inactivate enzyme. Then it was filtered by hands through two cheese cloths or muslin cloths. Then it was dried in an air ventilated oven for 24 hrs and ground to fine powder passing through sieve of size of 80 meshes and packed in airtight, moisture-proof bag at room temperature and ready to the extraction process. Fig 1(b) shows the finely ground orange peel powder.



Fig 1 (a) Orange peel (b) Orange peel powder

About 20 g peel powder was dissolved in 250 mL distilled water and pH was adjusted to 1.7 using 1N HCl. The mixture was heated in water bath at 90°C for 120 minutes. After 120 minutes, the mixture was cooled and was filtered through nylon cloth.

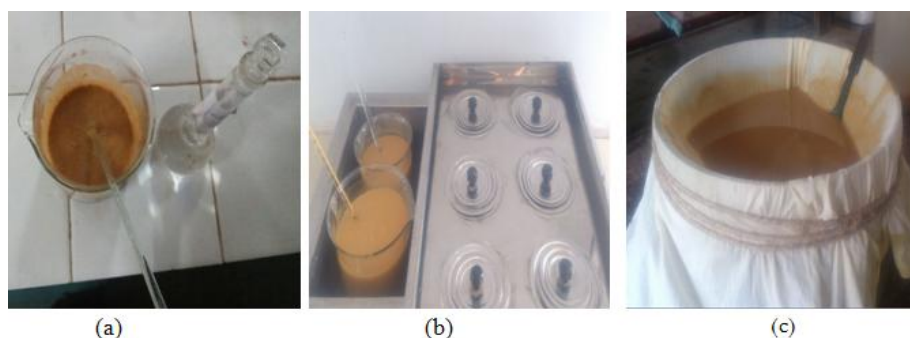


Fig 2 (a) Peel water mixture (b) Water bath heating (c) Filtration The filtrate was precipitated with 95% ethanol. The jelly pectin was filtered through whatmen No 1 filter paper and was washed with 95% ethanol. Then it was dried in an oven at 35°C. The pectin powder was then collected and the pectin yield was calculated [2].

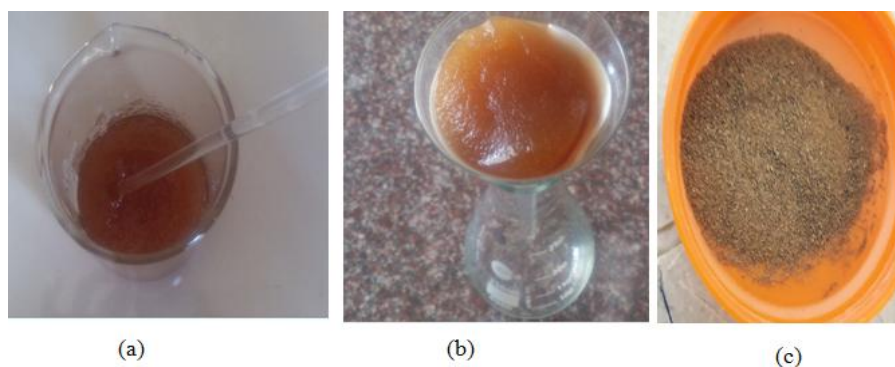


Fig 3 (a) Precipitation with ethanol (b) Jelly orange pectin (c) Orange pectin

The same extraction procedure is repeated using isopropyl alcohol instead of ethanol and the corresponding pectin yield was calculated.

2.3 Characterization of extracted pectin

Physical characteristics such as colour, solubility and gel forming ability were determined by visual observations. Equivalent weight, Methoxyl content and Anhyrouronic acid content were determined by titration with sodium hydroxide [3]. The degree of esterification was also determined by titration with NaOH [4]. Ash content was determined by ashing at 60 °C for 24 hours [5]. Moisture content was determined using an air oven method [5]. The morphological characteristics of pectin were obtained by SEM analysis. FTIR was used to

detect the chemical composition and functional groups present in the pectin. SEM and FTIR were analyzed from Sophisticated lab, Cochin University of Science and Technology, Ernakulum.

2.4 Coagulation- flocculation experiments using extracted pectin

Jar test was used to study the coagulation- flocculation process. Fig 4 shows conventional jar test apparatus used for studying the coagulation- flocculation process. Synthetic waste water was treated using coagulation flocculation process. FeCl_3 and orange pectin were used as coagulant and flocculant respectively.



Fig 4 Conventional jar test apparatus

The working volume for each sample was 1L. Desired pH was adjusted with 1M HCl or 1M NaOH. After adjusting the pH, coagulant was added followed by rapid mixing at 150 rpm for 3 minutes to homogenize the Fe^{3+} cation. Next, flocculant was added to the suspension at slow mixing, 30 rpm for 30 minutes. The suspension was then allowed to stand for 30 minutes for settling purposes. COD, phosphate, nitrate and turbidity of the supernatant solution were determined as per the procedures in standard methods. The corresponding removal efficiencies were found out.

2.5 Optimization of parameters

Optimization of parameters such as pH, coagulant dosage and pectin dosage were done using RSM in MINITAB software version 16. The ranges of these parameters were selected by doing random experimental trials.

2.6 Determination of settling characteristics of sludge

Settling characteristics of sludge can be analyzed using sludge volume index (SVI). The sludge volume index (SVI) is the volume in millilitres occupied by 1 g of a suspension after 30 min settling.

Real dairy waste water was treated using FeCl_3 and orange pectin. One litre of treated sample was allowed to settle for 30 minutes and then the supernatant was passed through a filter paper. The filter paper was dried and weighed to find suspended solids concentration. Then SVI can be calculated using (1):

$$\text{SVI} = \frac{\text{Settled sludge volume (mL/L)} \times 1000}{\text{Suspended solids (mg/L)}} \quad (1)$$

III. RESULTS AND DISCUSSIONS

3.1 Synthetic waste water preparation

Table1 shows the quantities of chemicals required for the preparation of synthetic waste water, which are obtained by trial and error method. Table2 shows the characteristics of real and synthetic waste water.

Table 1 Quantities of chemicals required for the preparation of synthetic waste water

Chemicals	Quantity (mg/L)
Glucose	180
Yeast	10
Milk powder	1500
Starch	30
Ammonium chloride	150
Dipotassium hydrogen phosphate	25
Potassium dihydrogen phosphate	18
Magnesium sulphate heptahydrate	100
Calcium carbonate	150
Sodium nitrate	50

Table 2 Characteristics of real and synthetic waste water

Parameter	Concentration of real waste water	Concentration of synthetic waste water
BOD	3100 mg/L	2900 mg/L
COD	4300 mg/L	4000 mg/L
Nitrate	252.1169 mg/L	303.901 mg/L
Phosphate	95.18 mg/L	60.24 mg/L
pH	6.7	6.4
TDS	541 ppm	446 ppm
Turbidity	470 NTU	311 NTU
Conductivity	1.05 mS	1.08S

3.2 Extraction

Table 3 shows the pectin yield obtained from orange peel using isopropyl alcohol and ethanol.

Table 3 Pectin yields from orange peel

Extraction solvent	Pectin yield (%)
Ethanol	30.799
Isopropyl alcohol	39.01

Comparing the two extraction solvents (ethanol and isopropyl alcohol), isopropyl alcohol gives highest pectin yield. Ethanol or isopropyl alcohol is added to the fruit extract, so as to increase the alcohol content. Due to the dehydration effect of alcohol, pectin precipitates as fibrous mass. It is due to the insolubility of pectin in alcohol. Since isopropyl alcohol is a secondary alcohol, its reactivity is greater than that of ethanol. Also, isopropyl alcohol dissolves more oil than ethanol. This may be the reason for the higher pectin yield from the extraction using isopropyl alcohol. Extraction from orange peel using isopropyl alcohol gives maximum pectin yield (39.01%).



3.3 Characterization

3.3.1 Physical characteristics

Physical characteristics of the extracted pectin include colour, solubility and gel forming ability. Table 4 shows the physical characteristics of pectin extracted from orange peel.

Table 4 Physical characteristics of extracted pectin

Colour	Brown
Solubility	<ul style="list-style-type: none"> • Not soluble in cold water. • Medium solubility in hot water
Gelling property	High

Colour of pectin is important as it indicate the purity of pectin. Pure form of pectin is usually light brown in colour. Factors such as surface contamination, environmental factors, types of fruits used and human error contribute to the changes in colour. Extracted pectin is dark brown in colour, which may be due to the presence of impurities. Pectin is less soluble in cold water and soluble in hot water. Orange pectin has only medium solubility in hot water. It may be due to the presence of impurities such as fibres in pineapple pectin. Pectin is a polymer having high gelling property. Orange pectin has high gelling property.

3.3.2 Chemical characteristics

Chemically, pectins are a mixture of complex polysaccharides, homogalacturonan being the main component. This is a linear polymer made up of repeated units of α -(1-4)-linked D-galacturonic acid, to form a long polygalacturonic chain. Pectin is capable of forming gels with sugars and acids under certain conditions. Gelling mechanism of pectin depends on various properties. Table 5 shows the chemical characteristics of the pectin extracted from orange peel.

Table 5: Chemical characteristics of pectin

Characteristics	
Equivalent weight	1250.00
Methoxyl content (%)	9.67
Total anhydrouronic acid (%)	68.99
Degree of esterification (%)	58.33
Moisture content (%)	6.09
Ash content (%)	1.01

Equivalent weight of pectin is an indicator of its gelly-forming ability, with high molecular weight pectin having better ability to form gels [6]. Equivalent weight of orange pectin is obtained as 1250.00, thus it is having higher gelling properties. Methoxyl content is an important factor in controlling the setting time of pectin, the sensitivity to polyvalent cations and their usefulness in the preparation of low solid gels and fibers. High methoxyl pectin (methoxyl content greater than 7%) forms gels in the presence of high sugar concentration, usually sucrose or fructose and at low pH; whereas low methoxyl pectins (less than 7.0%) can form gels with lower concentrations of sugars [6]. Methoxyl content of extracted pectin was obtained as 9.67%, which is greater than 7%. Hence extracted pectin can be considered as high methoxyl pectin and forms gels in the

presence of high sugar concentration. Pectin, which is a partly esterified polygalacturonide, contains 10% or more of organic materials composed of arabinose, galactose and other sugars. Anhydrouronic acid content indicates the purity of extracted pectin. AUA content of less than 65% indicate impurities due to the presence of proteins, starch and sugars in the precipitated pectin [6]. AUA content of extracted orange pectin was obtained as 68.99%. It is greater than 65%, hence it does not contains significant amount of impurities such as proteins, starch, sugar etc. Degree of esterification describes the rate of gel formation. Pectin having degree of esterification greater than 72% can be classified as rapid set pectin and pectin having degree of esterification between 58% and 65% can be classified as slow set pectin. DE of orange pectin was obtained as 58.33%. Thus orange pectin can be considered as slow set pectin. Low moisture content is necessary for pectin for safe storage as well as to inhibit the growth of microorganisms that can affect the quality due to the production of pectinase enzymes. Moisture content of orange pectin was obtained as 6.09 %. Lower ash content is a criterion governing the purity of pectin. Ash content of orange pectin was obtained as 1.01%.

3.3.3 SEM Analysis

Fig 5 shows the SEM image of extracted pectin powder. The structure was unique in shape, smooth and has more wrinkles on the surface.

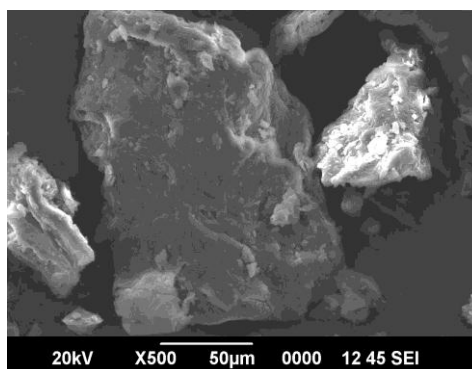


Fig 5 SEM image of extracted pectin

3.3.4 FTIR Analysis

FTIR spectra in the region between 4000-400 cm^{-1} identified the major chemical groups in the extracted pectin. The spectral data obtained were analyzed by comparing the FTIR spectra with the major absorptions in the IR spectra of peptic substances, which is shown in Table 6

Table 6 Band assignments of major absorptions in IR spectra of peptic substances

Frequency (cm^{-1})	Assignments
3600-3100	O-H stretching band (Acikgoz, 2010)
3000-2800	C-H stretching bands (Acikgoz, 2010)
1800-1200	Carboxylic groups (Acikgoz, 2010)
1750	Stretching C=O mode non-ionized methylated or protonated carboxyl ionization (Acikgoz, 2010)
1650-1600	Asymmetrical stretching vibrations due to the COO^- group of polygalacturonic acid (Acikgoz, 2010)
1450-1400	Symmetrical stretching vibrations due to the COO^- group of

	polygalacturonic acid (Acikgoz, 2010)
1200-1100	R-O-R or C-C bonds (Acikgoz, 2010)
1000-1104	Galacturonic acid (Acikgoz, 2010)
1070-1040	Carbohydrate backbone (Prabu and Natarajan, 2012)

Fig 6 shows the FTIR spectra of pectin extracted from orange peel. The peak centered at 3419 cm^{-1} indicates the presence of OH groups. The peaks in the region 1230 cm^{-1} and 1745 cm^{-1} show the presence of many carboxylic groups in the pectin molecules. A major band centered at 1647 cm^{-1} and a less intense band centered at 1441 cm^{-1} respectively shows asymmetrical and symmetrical stretching vibrations due to the COO group of polygalacturonic acid. The absorbances at 1018 cm^{-1} , 1072 cm^{-1} and 1103 cm^{-1} show the presence of galactoronic acid. The band centered at 1103 cm^{-1} and 1149 cm^{-1} may be due to R-O-R or C-C bonds in the pectin molecules. The bands at 2856 cm^{-1} and 2926 cm^{-1} indicates C-H stretching bands.

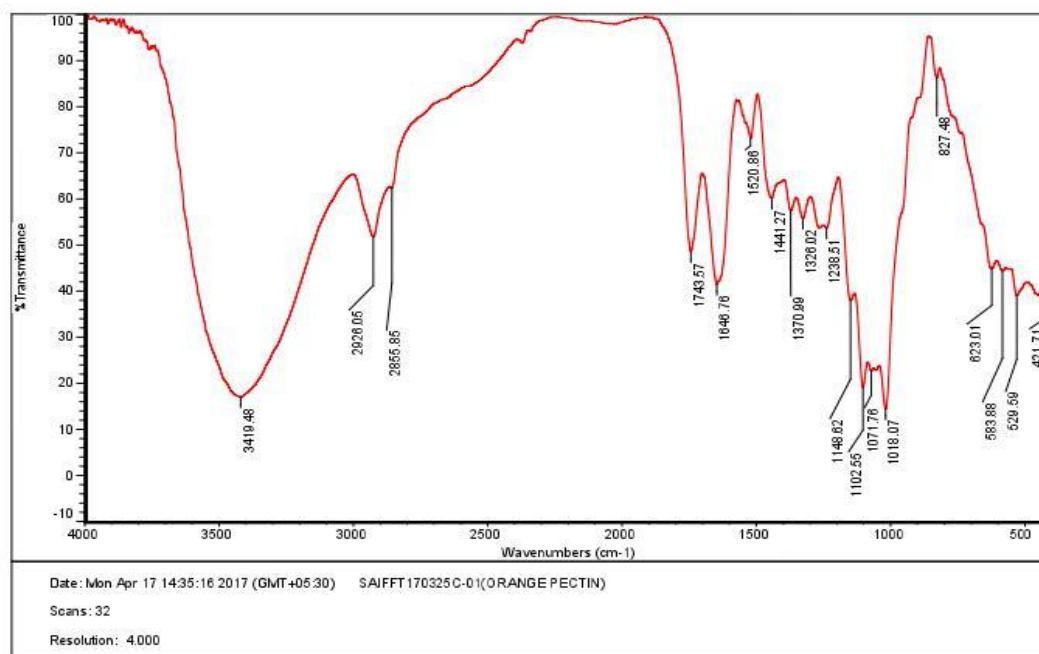


Fig 6 FTIR spectra of pectin extracted from orange peel

3.4 Coagulation-flocculation treatment

Ranges of parameters such as pH, FeCl₃ dosage and pectin dosage were determined by doing various initial trials, which is shown in Table 7.

Table 7 Levels of parameters for the treatment using orange pectin

Parameter	Low level	High level
pH	4	8
FeCl ₃ dosage (mg/L)	50	150
Pectin dosage (mg/L)	10	15

Table 8 shows the removal of COD, phosphate, nitrate and turbidity corresponding to the experimental runs obtained from the software.

Table 8 Results of experimental runs

Run order	pH	FeCl ₃ dosage (mg/L)	Pectin dosage (mg/L)	COD Removal (%)	Phosphate removal (%)	Nitrate removal (%)	Turbidity removal (%)
1	6	100	12.5	35	66.67	56.623	83.900
2	6	50	15.0	9.5	6.98	51.810	96.141
3	6	150	15.0	30	44.44	61.440	99.680
4	4	100	15.0	20	26.67	56.623	98.714
5	8	100	15.0	20	22.22	18.670	49.196
6	8	100	10.0	30	53.33	10.840	66.560
7	6	100	12.5	35	66.67	56.623	83.900
8	4	50	12.5	40	62.22	51.810	96.784
9	8	150	12.5	20	66.67	38.759	35.048
10	6	150	10.0	30	83.33	61.440	97.106
11	8	50	12.5	20	15.55	10.840	33.440
12	6	100	12.5	35	66.67	56.623	83.900
13	4	150	12.5	35	44.44	80.720	91.320
14	6	50	10.0	30	55.55	54.220	97.120
15	4	100	10.0	50	66.67	61.446	99.680

The optimum values of the experimental parameters (pH, FeCl₃ dosage and pectin dosage) for the removal of COD, phosphate, nitrate and turbidity are obtained by RSM using Minitab Software, version 6.1.

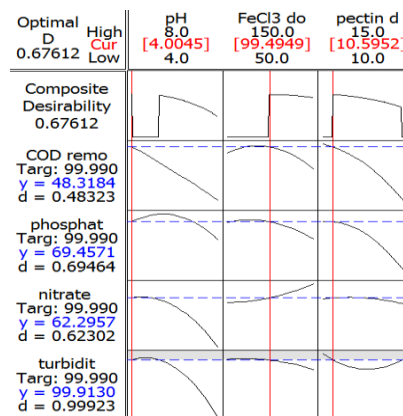


Fig 7 Optimization plot

Figure 7 shows the optimization plot obtained from the software. The optimum conditions obtained from Minitab are approximately pH=4.00, FeCl₃ dosage=99.49 mg/L and pectin dosage=10.59 mg/L. At the optimum conditions, COD removal, phosphate removal, nitrate removal and turbidity removal are 48.32%, 69.46%, 62.29% and 99.91% respectively.

pH is an important factor influencing the flocculating activity. Pectin gels in the presence of acids to form hydrogen-bonding and hydrophobic interactions, which reduces the electrostatic repulsions. At low pH, the high concentration of H⁺ causes formation of hydrogen-bonding and hydrophobic interactions. It leads to bridging

mechanism during flocculation. Hence a lower pH is favorable for effective coagulation-flocculation process (Chia et al, 2010). But there is an ideal range of pH for each of the coagulant. The pH range of FeCl_3 is 4 to 11. At a pH below 4, FeCl_3 is not active. Hence a pH of 4.00 was obtained as optimum pH from the software. By the coagulation flocculation process, dissolved materials can be precipitated and are removed by gravity. As a result a clear supernatant is obtained. Due to the removal of dissolved materials, there will be considerable removal of COD, phosphate, nitrate, turbidity etc.

Ferric chloride when added to water yields ferric and chloride ions. Ferric ions neutralize negatively charged particles and leads to agglomeration of the particles or it may combine with hydroxide ions to form ferric hydroxide which adsorbs particles, providing clarification of effluent. Low concentration of coagulant may affect the charge neutralization process and results in reduction of flocculation activity. High concentration of coagulant may lead to adverse effect where the surface of particle becomes positively charged. When this phenomenon occurs, bridging of particle during flocculation is difficult. Therefore an optimum coagulant dosage is required for effective flocculation (Chia et al, 2010). Hence a coagulant dosage of 99.49 mg/L was obtained as optimum dosage.

Pectin is added to the waste water in order to carry out the flocculation process. The effectiveness of flocculant adsorption depends on the amount of polymer adsorbed per unit area of the surface of the particles. At low concentration of flocculants, the flocculating activity is low. This is because the particle surface is not ready to induce any flocculation and very low adsorbent site is available for particle adhesion. At high flocculant concentration, the coagulation–flocculation process is not complete. The surface of particle is insufficient for polymer adsorption creating high competition among the flocculant. Hence an optimum concentration of flocculant is required for the effective flocculation (Chia et al, 2010). Therefore an optimum pectin dosage of 10.59 mg/L was obtained from the software.

3.5 Settling characteristics of sludge

Spellman's Standard Handbook for Wastewater Operators gives the settling characteristics of sludge corresponding to a particular SVI value. Table 9 shows the interpretation of SVI results, which are given by spellman's standard handbook.

Table 9 Interpretation of SVI results

SVI Range	Expected condition
< 100mL/g	Old sludge; possible pin floc; increasing effluent turbidity
100mL/g to 250mL/g	Normal operation; good settling; low effluent turbidity
> 250mL/g	Bulking sludge; poor settling; high effluent turbidity

The obtained SVI value is 625.00 mL/g, which is above 250 mg/L. Thus the sludge is having poor settling characteristics and high effluent turbidity.

IV. CONCLUSIONS

The study investigated the performance of biopolymeric flocculant (pectin) extracted from orange peel to treat dairy waste water. Extraction performances of two solvents (ie isopropyl alcohol and ethanol) were compared. The results revealed that Isopropyl alcohol is more effective for the extraction of pectin than ethanol. Extraction from orange peel using isopropyl alcohol gave maximum pectin yield ie 39.01%. Chemical composition of pectin was analyzed by using titrimetric method. The results revealed that orange pectin has higher gelling properties. Extracted pectin can be considered as high methoxyl pectin and forms gels in the presence of high sugar concentration. Detailed characterization of extracted pectin was carried out using FTIR analysis and SEM analysis. FTIR analysis confirms the presence of hydroxyl groups and polygalacturonic acid in the pectin molecules. The SEM image gave a smooth morphological topography of pectin molecules.

Synthetic dairy waste water was treated by coagulation-flocculation method using FeCl_3 as coagulant and the extracted pectin as flocculant. The effectiveness of orange pectin to treat dairy waste water was analyzed. The results showed that orange pectin can be used for the pre-treatment of dairy waste water. It significantly reduces COD, phosphate, nitrate and turbidity from the dairy waste water.

Response surface methodology was carried out using Box Behnken design to find the optimum pH, coagulant dosage and flocculant dosage to maximize COD removal, phosphate removal, nitrate removal and turbidity removal. It can create a set of experimental runs that reduce the number of runs needed to optimize the operating conditions. The optimum conditions obtained from Minitab are approximately pH=4.00, FeCl_3 dosage=99.49 mg/L and pectin dosage=10.59 mg/L. At the optimum conditions, COD removal, phosphate removal, nitrate removal and turbidity removal are 48.32%, 69.46%, 62.29% and 99.91% respectively.

Real dairy waste water was treated under obtained optimum conditions of pH, coagulant dosage and pectin dosage. Setting characteristics of sludge was evaluated based on Sludge volume index. The results revealed that the sludge is having poor settling characteristics and high effluent turbidity.

V. ACKNOWLEDGMENT

The authors wish to thank the Govt. Engineering College, Trichur, Thrissur, Kerala where the study was conducted.

REFERENCES

- [1] M. Nazeri Salleh, A. Idris, M.N.M. Yunus, and S. Kathiravale, Physical and chemical characteristics of solid waste in Kuala Lumpur, Malaysia, 2009.
- [2] S.A.E.I. Nawawi and F.R. Shehata, Extraction of Pectin from Egyptian Orange Peel: Factors Affecting the Extraction, 20, 1987, 281-290.
- [3] E.Girma and T.Worku, Extraction and Characterization of Pectin from Selected Fruit Peel Waste, *International Journal of Scientific and Research Publications*, 6(2), 2016, 2250-3153.
- [4] L.Qin, C.Ling and Y, Extraction and Characterization of Pectin from Passion Fruit Peels, *Agriculture and Agricultural Science Procedia*, 2, 2014, 231 – 236.



- [5] V.O. Aina, M. M. Barau, O.A. Mamman, A. Zakari, H. Haruna, M.S. Umar and Y.B. Abba, Extraction and Characterization of Pectin from Peels of Lemon (*Citrus limon*), Grape Fruit (*Citrus paradisi*) and Sweet Orange (*Citrus sinensis*), *British Journal of Pharmacology and Toxicology*, 3(6), 2012, 259-262.
- [6] V.A.Vaclavik and E.W. Christian, Essentials in food science. 3rd ed. USA: Springer Science+Business, 2008.
- [7] H. Chia, Ismail, F.M.Abbas and Morad, Reuse of Fruit Waste as Biopolymeric Flocculant and Optimizing Turbidity Reduction: Comparison Study with Industrial Flocculant, *Journal of environmental engineering © asce*,136(11), 2010, 1267-1276.