

# The Study of Soluble Microbial Products in Submerged Membrane Bioreactor (Part 1: Residential Quarter Wastewater)

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## Abstract

The objective of present study is to study impact of sludge retention time (SRT) and hydraulic retention time (HRT) on Soluble Microbial Products (SMP), Proteins and Carbohydrates for residential quarter wastewater. The experiments have been performed on laboratory-scale hollow fiber submerged membrane bioreactor (MBR) for two different seasons. The two seasons considered was dry season i.e. summer season and another is wet season i.e. rainy season. For residential quarter wastewater, the SMP is varied from 7.9 to 8.6 mg/l. Protein is varied from 4.0 to 5.8 mg/l. Carbohydrate is varied from 2.4 to 4.25mg/l and C/P ratio is varied from 0.47 to 1.20. It is observed that SMP and Carbohydrates are increase with increase in SRT and HRT and Proteins decrease.

#### Introduction

SMP can be defined as the pool of organic compounds that are released into solution from substrate metabolism (usually with biomass growth) and biomass decay [1]. Thus, SMP can be subdivided into two categories [2,3] : substrate-utilisation-associated products (UAP), which are produced directly during substrate metabolism, and biomass-associated products (BAP), which are formed from biomass, presumably as part of decay.SMP is very heterogeneous, in which a variety of polymeric materials have been found: carbohydrates, proteins, lipids and nucleic acids. In this work, however, the sum of total carbohydrates and proteins was considered to represent the total amount of SMP because these are the dominant components typically found in SMP. The effect of various process parameters on the production, accumulation and elimination of SMP is of considerable concern for researchers and engineers [4, 5]. The organic matter (COD) in the wastewater contains a variety of soluble organic and non or slowly biodegradable substrates in the influent as well as intermediate substrates and in the end products [6]. Soluble Microbial Products (SMPs) have proved to be the major components of the soluble organic matter in effluents from biological treatment process [7]. Many authors used he concept of microorganisms producing organic material during growth and death is well accepted the term SMP without precise definition. This is partly due to the difficulty in identifying SMP experimentally, but also due to the complexities of effluent composition [1]. SMP can be defined as the pool of organic compounds that result from substrate metabolism (usually with biomass) and biomass decay [8]. Based on the bacterial phase, SMP grouped into two different categories i.e utilization associated products (UAP)



and biomass associated products (UAP). SMP that are associated with substrate metabolism and biomass growth and are produced at a rate proportional to the rate of substrate utilization is named as UAP.SMP that are associated with biomass decay and are produced at a rate proportional to the concentration of biomass is named as BAP [9]. SMP comprise a wide range of high and low molecular weight compounds including proteins, polysaccharides, humic and fulvic acids, nucleic acids, enzymes and structural compounds[10] and are therefore difficult to measure. In spite of the ambiguity related to SMP, interest in this area has been growing over the years due to the progressively stringent discharge criteria being implemented worldwide. Membrane bioreactor (MBR) technology is a steadily growing wastewater treatment solution capable of generating high-quality effluent by retaining solids and SMP [11]. The accumulation of SMP has been shown to adversely affect membrane flux, metabolic activity of activated sludge, and nitrification [12]. Few studies have examined accumulation, molecular weight (MW) distributions and fate of SMP in aerobic MBRs over a period of only 300 days or less [12,6]. Both studies reported an accumulation in SMP followed by a drop in aerobic MBR at an SRT of 20 days when operating at total suspended solids (TSS) concentrations of 3-8.5 g /liter. Molecular weight distribution (MWD) of SMP showed that the effluent contained compounds with a board spectrum of molecular weight (< 0.5 - >50 kDa) and that a greater amount of high molecular weight compounds were found in many biological effluents than in the influent. This means that SMP may be more toxic than that the original organic compounds present in the wastewater. Some SMP have also been found to be inhibitory to nitrification [6].

**Maximous et al (2009)** assessed the relationship between membrane hydrophobicity and fouling properties; polysaccharide and protein rejection characteristics in a membrane bioreactor (MBR) system, two ultrafiltration membranes with different hydrophilic/hydrophobic properties were tested using activated sludge and soluble microbial products (SMPs). The initial and pseudo steady-state fouling rates for sludge filtration by both membranes were higher than those of SMP filtration suggesting that the suspended solids exerted a negative impact on membrane fouling rate. A modification of the resistance-in-series model was applied and the results showed that cake resistance was the main fouling mechanism for both membranes. The hydrophobic membrane (poly-ethersulfone) showed greater polysaccharide and protein rejection and consequently lower flux than the hydrophilic membrane (regenerated cellulose). The relatively higher cake resistance of the poly-ethersulfone membrane rationalizes the increased solute rejection. The comparable observed pseudo steady-state fouling rates and permeability with the two membranes appears to dispel any advantages of hydrophilic membranes for sludge filtration [21]. Soluble microbial products (SMP) are the pool of organic compounds originating from microbial growth



and decay, and are usually the major component of the soluble organic matters in effluents from biological treatment processes [13].

In this work, SMP in activated sludge were characterized, fractionized, and quantified using integrated chemical analysis and mathematical approach. The utilization-associated products (UAP) in SMP, produced in the substrate-utilization process, were found to be carbonaceous compounds with a molecular weight (MW) lower than 290 kDa which were quantified separately from biomass-associated products (BAP). The BAP were mainly cellular macromolecules with an MW in a range of 290–5000 kDa, and for the first time were further classified into the growth-associated BAP (GBAP) with an MW of 1000 kDa, which were produced in the microbial growth phase, and the endogeny-associated BAP (EBAP) with an MW of 4500 kDa, which were generated in the endogenous phase. Experimental and modeling results reveal that the UAP could be utilized by the activated sludge and that the BAP would accumulate in the system. The GBAP and EBAP had different formation rates from the hydrolysis of extracellular polymeric substances and distinct biodegradation kinetics. This study provides better understanding of SMP formation mechanisms and becomes useful for subsequent effluent treatment.

**Pan et al (2010)** measured the accumulation and characteristics of soluble microbial products (SMP) in the mixed liquor and the effluent of the membrane bioreactor (MBR). It was found that the concentration of SMP decreased when the SRT was increased from 10 days to 30 days, and then stabilized as SRT was increased to 60 days. The molecular weight (MW) distributions of SMP indicated that the SMP of larger MW (>30kDa) was the most abundant fraction in the MBR. The similar MW distributions of SMP in the mixed liquor and effluent implied that membrane fouling due to SMP in the initial slow fouling stage was not due to size sieving. After the MBR was operated for a period of time, only the SMP of relatively large MW (>30kDa) was detected in the mixed liquor. The result indicated that size sieving of SMP occurred only after a cake layer was formed on the membrane surface although the effect was not significant and only worked on larger molecules. The accumulation of hydrophilic components of SMP in the mixed liquor of the bioreactor suggested that the hydrophilic fraction (in carbohydrates) could be the major cause for membrane fouling [15]. Soluble microbial products (SMPs) present a major part of residual chemical oxygen demand (COD) in the effluents from biological wastewater treatment systems, and the SMP formation is greatly influenced by a variety of process parameters.

**Xu et al (2011)** used the response surface methodology (RSM) coupled with grey relational analysis (GRA) method to evaluate the effects of substrate concentration, temperature, NH<sub>4</sub> concentration and aeration rate on the SMP production in batch activated sludge reactors. Carbohydrates were found to be the major component of SMP, and the influential priorities of these factors were: temperature > substrate concentration > aeration rate > NH<sub>4</sub> concentration. On the basis of the response surface methodology (RSM) results, the interactive effects of these factors on the SMP formation were evaluated, and the

optimal operating conditions for a minimum SMP production in such a batch activated sludge system also were identified. These results provide useful information about how to control the SMP formation of activated sludge and ensure the bioreactor high-quality effluent [15].

**Yao et al (2011)** investigated the comprehensive fouling propensity associated with the change of soluble microbial products (SMP), especially the concentration of SMP and the ratio of protein to polysaccharide (PN/PS) in a lab scale submerged microfiltration MBR. Results showed that the higher ratio of PN/PS induced less irreversible fouling and improved the interaction of protein and polysaccharide to form cake layer. The rejection efficiency of major components in SMP also increased with higher PN/PS ratios. Fouling mechanisms altered from combination of intermediate pore blocking and cake formation at initial stage to cake formation on the membrane surface during long-term operation. Moreover, the irreversible fouling resistance was found to be proportional to the concentration of SMP. Lower concentration of SMP and higher PN/PS ratio should be an effective strategy in releasing membrane fouling [16].

**Tian et al (2011)** conducted a series of stirred dead-end filtration tests to investigate the fouling potential of BAP (biomass-associated products) and UAP (utilization-associated products), showing that UAP had higher modified fouling index with a value of  $1.63 \times 10^4$  s/L<sup>2</sup>. Proteins were mainly accumulated in UAP and accounted for 45.3%, while the proportion of proteins in BAP was only 12.1%. Due to the membrane interception, the permeate concentrations of UAP and BAP decreased largely: proteins decreased to near zero and polysaccharides were effectively retained ranging from 26.6% to 46.8%. Excitation–emission matrix analysis showed that humic-like and fulvic-like compounds had lower retention than proteins. The observation of fouled membrane by Fourier transform infrared spectroscopy demonstrated that membrane foulants were dominated by proteins and polysaccharides. Modeling work well indicated that the main fouling mechanisms for BAP and UAP filtration were cake filtration and complete blockage, respectively, further confirming that UAP had higher fouling potential than BAP [17].

The objective of present study is to find out the effect of SMP on performance of MBR.

# **Material and Methods**

A Submerged MBR assembly (100 L/day in Capacity) was fabricated to investigate applicability of membrane technology for Indian conditions. The feed substrates for the MBR reactor were the hospital wastewater collected from the drainage of a hospital on regular basis. For the reactor assembly, re-denitrification scheme (denitrification tank with a volume of 36 L) had been adopted for nitrogen removal, and a membrane module was immersed in the nitrification tank (volume 49 L). The permeate extraction regime was an alternate relaxation (2 min) followed by a suction phase (8 min). Aeration was carried out at the bottom of filtration module using a coarse diffuser in order to reduce fouling processes by turbulent flow generated along membranes. Mechanical cleaning was achieved by means of air bubble

blowing at the bottom of the module. Permeate was withdrawn under suction from the membrane using a piston pump. To avoid the entrainment of air, nitrogen gas was introduced to maintain anoxic condition.

The seed biomass was initially acclimatized to aerobic and subsequently to anoxic conditions in batch mode. The 77 L reactor was subsequently seeded at a ratio 4:1. The reactor was operated under ambient conditions for which the temperature ranged between 29 to  $31^{\circ}$ C (Average of  $30^{\circ}$ C) for the entire operation period of three months. The hydraulic residence time (HRT) was varied as 4, 6 and 8 h and the SRT in successive tests was set at 10, 20 and 30 days, respectively.

SMP quantification was made on the sludge supernatant that had been obtained by centrifugation at 2000 rpm for 20 min, and on the suspended solid. The SMP from the suspended solid were extracted by addition of 2N NaOH at  $4^{\circ}$ C for 4 h. The extracted solutions were then centrifuged at 8,000 rpm for 20min and filtrated on a 0.2µm membrane. SMP were quantified in influent and permeate samples. Proteins were measured by spectrophotometric methods.

SMP can accumulate on the membranes or penetrate into membrane pores. Accumulation and detachment of membrane foulants are determined by particle convection towards the membrane surface and the back transport rate of the deposited particles from membrane surface into the bulk. It is difficult to control the back transport of colloids and solutes only by enhancing aeration intensity due to the small size of these substances. The control of SMP concentration in MBRs is crucial. In general, the control of SMP can be achieved by two approaches: adjustment of operation parameters (i.e., SRT, HRT, DO concentration, temperature, aeration).SMP is a complex mixture of macromolecules including polysaccharides, proteins, nucleic acids, humic acids, etc. In this study, the total SMP is defined as the sum of carbohydrates and proteins because they are the main components of SMP [18]. To analyse the SMP in biomass suspensions, 100 mL of mixed liquor from activated sludge was collected from MBR. The samples were filtered through 0.45-mm PTFE filter to separate the residual biomass, and the filtrates were subjected to SMP analysis. The SMP was calculated as the sum of the following three components:

# Total SMP = carbohydrates + proteins

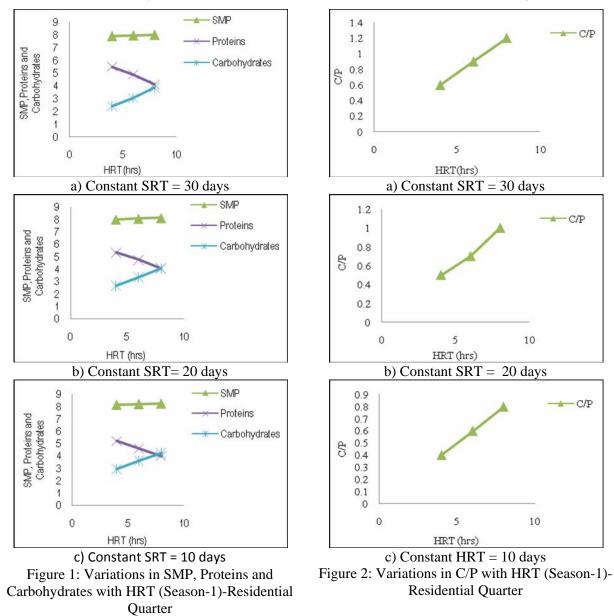
The phenol-sulfuric acid method [19] and the Lowry method (calorimetrically using Bicinchoninic Micro Acid protein assay)[20] were used for determination of the concentrations of carbohydrates and proteins, respectively. Glucose and bovine serum albumin (BSA) were used as standards for the measurements of carbohydrates and proteins, respectively [4, 16, 21]. The acetate concentration was measured using a gas chromatograph. The total SMP was determined from the difference as follows [5, 13, 17]: *Total SMP* (*as COD*) = *So* lub*le COD* – 1.07 \* *CH*<sub>3</sub>*COONa* 



## **Results and Discussion**

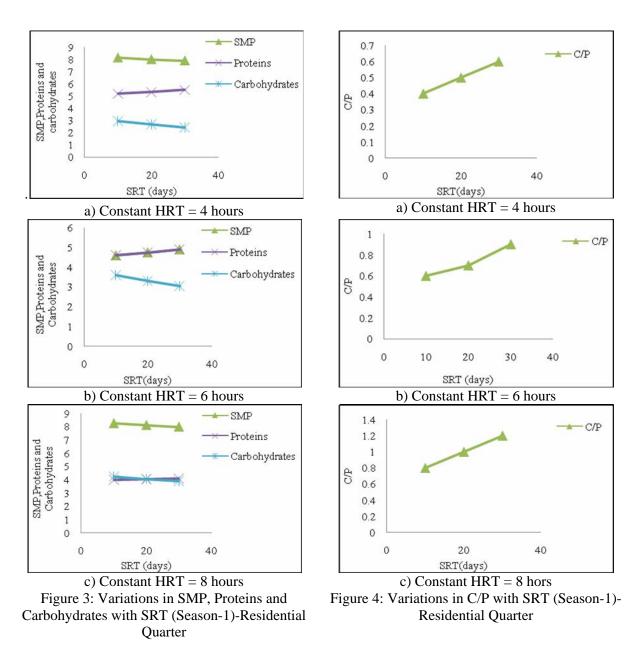
## Variation of SMP, Protein, Carbohydrates and C/P ratio with SRT

The variation in SMP, Proteins and carbohydrates with different SRTs and constant HRT is shown in figure 3 for residential quarter wastewater in the summer season and that for rainy season, variation in SMP, Proteins and carbohydrates with different SRTs and constant HRT is illustrated in figure 7



Again, the variation in C/P ratio with different SRTs and constant HRT is shown in figure 4 for residential quarter wastewater in the summer season and that for rainy season, variation in C/P ratio with different SRTs and constant HRT is illustrated in figure 8.

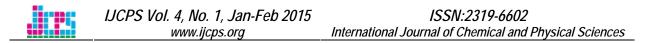




# Summer Season

For **residential quarter wastewater**, the variation in SMP, Proteins and Carbohydrates are illustrated in figure 3 and variation in C/P ratio is illustrated in figure 4.

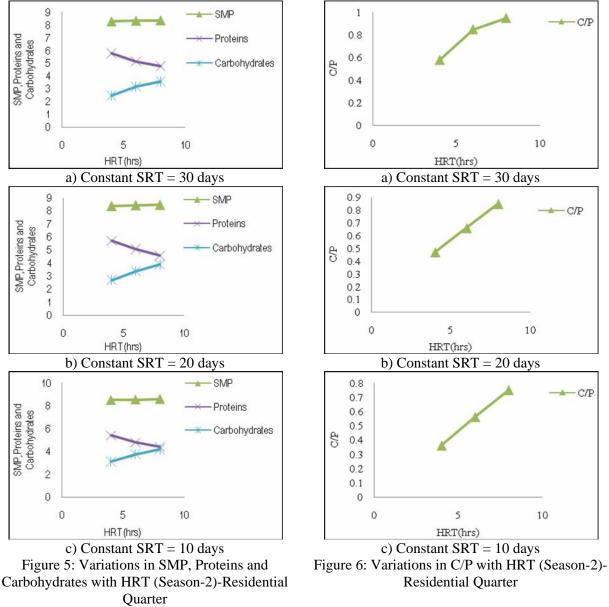
During HRT of 4 hours, SMP, Proteins and Carbohydrates in activated sludge vary from 8.15 to 7.90 mg/l, 5.2 to 5.55 mg/l and 2.95 to 2.4 mg/l respectively for SRT change from 10 to 30 days. For HRT of 6 hours, SMP, Proteins and Carbohydrates in activated sludge vary from 8.21 to 7.95 mg/l, 4.6 to 4.9 mg/l and 3.61 to 3.05 mg/l respectively for SRT change from 10 to 30 days. With regard to HRT of 8 hours, SMP, Proteins and Carbohydrates in activated sludge vary from 8.25 to 7.99 mg/l, 4.00 to 4.1 mg/l and 4.25 to 3.89 mg/l respectively for SRT change from 10 to 30 days. However, During HRT of 4 hours, C/P



ratio in activated sludge varies from 0.4 to 0.6 for SRT change from 10 to 30 days. For HRT of 6 hours, C/P ratio in activated sludge varies from 0.6 to 0.9 for SRT change from 10 to 30 days. With regard to HRT of 8 hours, C/P ratio in activated sludge varies from 0.8 to 1.2 for SRT change from 10 to 30 days

## **Rainy Season**

For **residential quarter wastewater**, the variation in SMP, Proteins and Carbohydrates are illustrated in figure 7 and variation in C/P ratio is illustrated in figure 8.

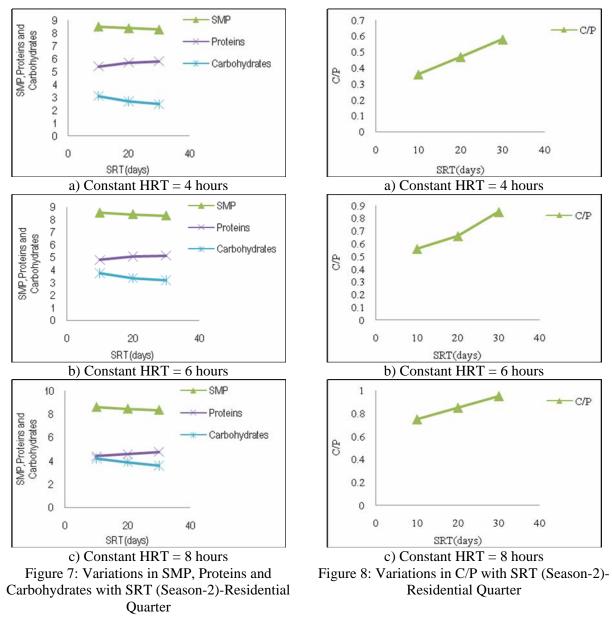


However, During HRT of 4 hours, C/P ratio in activated sludge varies from 0.36 to 0.58 for SRT change from 10 to 30 days. For HRT of 6 hours, C/P ratio in activated sludge varies from 0.56 to 0.85 for SRT change from 10 to 30 days. With regard to HRT of 8 hours, C/P ratio in activated sludge varies from 0.75 to 0.95 for SRT change from 10 to 30 days.



## Variation of SMP, Protein, Carbohydrates and C/P ratio with HRT

The variation in SMP, Proteins and carbohydrates with different HRTs and constant SRT is shown in figure 1 for residential quarter wastewater in the summer season and that for rainy season variation in SMP, Proteins and carbohydrates with different HRTs and constant SRT is illustrated in figure 5.



Again, the variation in carbohydrates to proteins ration (C/P ration) with different HRTs and constant SRT is shown in figure 2 for residential quarter wastewater in the summer season and that for rainy season variation in C/P ratio with different HRTs and constant SRT is illustrated in figure 6.



### Summer Season

For **residential quarter wastewater**, the variation in SMP, Proteins and Carbohydrates are illustrated in figure 1 and variation in C/P ratio is illustrated in figure 2.

During SRT of 10 days, SMP, Proteins and Carbohydrates in activated sludge vary from 8.15 to 8.25 mg/l, 5.2 to 4.00 mg/l and 2.95 to 4.25 mg/l respectively for HRT change from 4 to 8 hours. For SRT of 20 days, SMP, Proteins and Carbohydrates in activated sludge vary from 8.00 to 8.12 mg/l, 5.33 to 4.06 mg/l and 2.67 to 4.06 mg/l respectively for HRT change from 4 to 8 hours. With regard to SRT of 30 days, SMP, Proteins and Carbohydrates in activated sludge vary from 7.90 to 7.99 mg/l, 5.5 to 4.1 mg/l and 2.4 to 3.89 mg/l respectively for HRT change from 4 to 8 hours. However, During SRT of 10 days, C/P ratio in activated sludge varies from 0.5 to 1.0 for HRT change from 4 to 8 hours. With regard to SRT of 20 days, C/P ratio in activated sludge varies from 0.6 to 1.2 for HRT change from 4 to 8 hours.

#### **Rainy Season**

For **residential quarter wastewater**, the variation in SMP, Proteins and Carbohydrates are illustrated in figure 5 and variation in C/P ratio is illustrated in figure 6.

During SRT of 10 days, SMP, Proteins and Carbohydrates in activated sludge vary from 8.50 to 8.60 mg/l, 5.4 to 4.4 mg/l and 3.1 to 4.2 mg/l respectively for HRT change from 4 to 8 hours. For SRT of 20 days, SMP, Proteins and Carbohydrates in activated sludge vary from 8.37 to 8.46 mg/l, 5.69 to 4.57 mg/l and 2.68 to 3.89 mg/l respectively for HRT change from 4 to 8 hours. With regard to SRT of 30 days, SMP, Proteins and Carbohydrates in activated sludge vary from 8.27 to 8.35 mg/l, 5.8 to 4.77 mg/l and 2.47 to 3.58 mg/l respectively for HRT change from 4 to 8 hours. However, During SRT of 10 days, C/P ratio in activated sludge varies from 0.36 to 0.75 for HRT change from 4 to 8 hours. For SRT of 20 days, C/P ratio in activated sludge varies from 0.47 to 0.85 for HRT change from 4 to 8 hours. With regard to 8 hours. With regard to 8 SRT of 30 days, C/P ratio in activated sludge varies from 0.47 to 0.85 for HRT change from 4 to 8 hours. With regard to 8 hours.

## Conclusion

The soluble microbial products (SMP) include proteins and carbohydrates which play important role in membrane fouling. As SMP in system is increased the accumulation of bioparticles on the membrane surface and pore blocking of the membrane are increased that results into increased membrane fouling.

The concentration of SMP is increases with increase in SRT and HRT. In addition, as SRT and HRT increases concentration of Proteins decrease and carbohydrates concentration increase. Presence of SMP found more in Hospital Wastewater as compare to residential quarter wastewater. It may be due to presence organic compound in hospital wastewater. SMP found more in wet season as compare to dry season as the temperature is more suitable for growth of micro organism in wet season.



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