Effect of carbon to nitrogen ratio on the composition of microbial extracellular polymers in activated sludge

B. Durmaz and F.D. Sanin*
Department of Environmental Engineering, Middle East Technical University, 06531 Ankara, Turkey

Abstract Effect of carbon to nitrogen ratio (C/N) on the sludge extracellular polymer composition is studied in synthetically fed semi-continuous reactors with 8 days of sludge age. Results show that C/N ratio influences the relative distribution of polymer carbohydrate and protein. At low C/N ratio of 5, polymer extracts have high protein and low carbohydrate content. As the C/N ratio is increased to 17.5 and then to 40, carbohydrate concentration increases sharply and protein concentration decreases.

Keywords Activated sludge; carbohydrates; C/N ratio; extracellular polymers; proteins

Introduction
It is known that biofloc formation occurs due to the presence of extracellular polymers (Pavoni et al., 1972; Horan and Eccles, 1986; Bruus et al., 1992; Urbain et al., 1993). Over the past few decades there has been much research interest on the function, structure and properties of extracellular polymers. It has been reported that the main components of this polymer network holding the microbial system in activated sludge in the form of a floc are carbohydrates, proteins, nucleic acids and lipids (Pavoni et al., 1972; Horan and Eccles, 1986; Brown and Lester, 1980). Extracellular carbohydrates are produced by most bacteria out of the cell wall with the purpose of providing cells with the ability to compete in a variety of natural environments, providing a mode for adhesion to surfaces and protection of pathogenic bacteria against phagocytosis (Neidhardt et al., 1990). Polysaccharides are the only component that are synthesized extracellularly for a specific function. On the other hand, proteins, lipids and nucleic acids can exist in the extracellular polymer network due to the excretion of intracellular polymers or as a result of cell lysis.

There have been confusing reports on the composition of extracellular polymers, especially on the carbohydrate versus protein concentrations in the extracts. Some researchers have identified proteins as the predominant component of the activated sludge extracellular polymers (Goodwin and Forster, 1985; Frolund et al., 1996; Higgins and Novak, 1997), others report that carbohydrates are the predominant component (Pavoni et al., 1972; Horan and Eccles, 1986; Forster and Clarke, 1983). Some of these results came from work with treatment plant activated sludges and others were from lab-grown microorganisms. Part of the discrepancy is believed to originate from the extraction method whereas part may be explained by the wastewater feed composition. Activated sludge systems treating municipal wastewaters mostly operate under carbon limited conditions. The typical municipal wastewater composition has a carbon to nitrogen (C/N) ratio that varies between 25/1 to 20/1; carbon defined in terms of COD and nitrogen defined in terms of ammonium ion (Gaudy and Gaudy, 1980). Carbon is needed for growth and energy purposes and it is associated with cellular materials and intracellular and extracellular polysaccharide production. Nitrogen in the cells is associated with protein and nucleic acids (Norland et al., 1995).

Since carbon and nitrogen have specific purposes and uses in the cells, C/N ratio becomes important in determining total carbohydrate and total protein content of the cells. Besides, there are indications that the C/N ratio influences the amount and composition of...
extracellular polymers. Variations of extracellular polymer production with nutrient conditions have been mostly studied in pure culture systems and as Bengtsson (1991) indicates, highest yields of extracellular polysaccharides are obtained when the carbohydrate is present in excess and the growth is limited by the available nitrogen, phosphorus or sulfur source. Allison and Sutherland (1987) note that when bacteria were grown under glucose limiting conditions only trace amounts of carbohydrate could be detected associated with the attached cells. Harris and Mitchel (1973) have argued that under carbon limited growth, the synthesis of extracellular polymers would be unlikely, however even these microorganisms are known to be able to aggregate. This is thought to be due to the excretion of intracellular polymers, or polymers becoming extracellular as the result of partial cellular lysis. Wu et al. (1982) examining the effect of C/N ratio on the filterability of lab-grown activated sludge samples, reported that nitrogen restricted activated sludge seems to be high in carbohydrate content but low in protein content. Even though what is being reported is the total sludge protein and carbohydrate, they also report that under nitrogen restricted conditions the sludge solids accumulate a considerable amount of capsulated cell materials on the cell surface based on their microscopical analysis. C/N ratio seems to be important in the aggregation of microorganisms as noted by Burdman et al. (2000). They noted that media with low C/N ratio tend to promote a dispersive growth, whereas in the presence of a medium with high C/N ratio the cells tend to aggregate and flocculate.

Most of the available information in the literature on the effect of C/N ratio on the extracellular polymer production and composition is either with pure culture bacteria or in the form of some indirect indications that do not aim to quantify the polymers produced at specific C/N ratios. Therefore the purpose of this research was to investigate the effect of C/N ratio on the production and composition of extracellular polymers of lab-grown mixed activated sludge cultures. The results are expected to contribute to clarifying the issue of what kind of growth conditions stimulate the production of extracellular carbohydrates and proteins.

**Materials and methods**

**Reactor operation and production of microorganisms**

Mixed culture bacteria grown in semi-continuous reactors were used during the experiments. The microbial seed was obtained from the primary settling tank effluent from the city of Ankara wastewater treatment plant. The reactors were fed synthetically with the medium, composition of which is given in Table 1.

Reactors had a working volume of 2 litres and were operated at a mean cell residence time (MCRT) of 8 days. The temperature of the system was kept at 20°C, pH was 7.0 ± 0.2 and the DO concentration was kept at a minimum of 3 mg/L.

**Table 1** Synthetic feed medium composition

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>935</td>
</tr>
<tr>
<td>Peptone</td>
<td>200</td>
</tr>
<tr>
<td>K2HPO4</td>
<td>600</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>300</td>
</tr>
<tr>
<td>NH4Cl</td>
<td>225</td>
</tr>
<tr>
<td>MgSO4.7H2O</td>
<td>112.5</td>
</tr>
<tr>
<td>FeSO4.7H2O</td>
<td>3.75</td>
</tr>
<tr>
<td>ZnSO4.7H2O</td>
<td>3.75</td>
</tr>
<tr>
<td>MnSO4.7H2O</td>
<td>3.75</td>
</tr>
<tr>
<td>CaCl2</td>
<td>15</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>180</td>
</tr>
</tbody>
</table>
Three sets of reactors were operated under three different carbon (C) to nitrogen (N) ratio. First C/N ratio was selected as 17.5 (in terms of the ratio of COD to NH₄). This set of reactors (replica reactors 1 and 2) was operated to represent the typical operational conditions in activated sludge systems treating municipal wastewaters. These reactors were fed with the synthetic medium given in Table 1. The second set of reactors (reactors 3 and 4) was operated at a C/N ratio of 5 to represent a carbon-limited situation. To adjust the amount of carbon, the synthetic medium given in Table 1 was modified and the glucose amount in the feed was decreased to one-fourth amount. The third set of reactors (reactors 5 and 6) was operated at a C/N ratio of 40 representing a nitrogen-limited situation. The carbon content of these reactors was again adjusted by modifying the glucose amount in the feed given in Table 1. The average ammonia nitrogen in the feed was 59 mg/L with the total nitrogen being equal to 62 mg/L. So most of the nitrogen provided is in the form of ammonia nitrogen.

Handling of microorganisms and extracellular polymer extraction
Reactors operated under the above listed conditions were first brought to steady state. Steady state in the reactors was followed by measuring the effluent COD concentrations as well as MLSS and MLVSS concentrations. Leveling of these parameters measured during 4–6 successive days indicated that the steady state had been reached. Once the steady state was reached, polymer extraction experiments were conducted on 250 mL of daily waste sludge from each reactor.

A cation exchange resin was used to extract the extracellular polymers from the mixed culture microorganisms as suggested by Frolund et al. (1996). Dowex 50X8 (20–50 mesh) strongly acidic cation exchange resin (CER) in sodium form was supplied from Fluka and a method similar to the one used by Frolund et al. (1996) was applied.

First the time dependency of polymer extraction with CER technique was tested at a fixed CER concentration, next the effect of applied CER dose on the amount of polymer extracted was investigated. Prior to any CER extraction, sludge samples removed from the reactors were washed by centrifuging the samples at 3,500 rpm for 15 minutes. The centrates were discarded and the pellets were resuspended in phosphate buffer saline (PBS) solution (NaCl: 4 g/L, KCl: 0.1 g/L, KH₂PO₄: 0.06 g/L, Na₂HPO₄: 0.455 g/L) and polymer extraction tests were applied immediately. CER to be used in extractions was washed for an hour by mixing it in the PBS solution, and then dried at room temperature. Polymer extractions were done by using a standard jar test apparatus operated at a stirring speed of 120 rpm. Each extraction test was accompanied by two controls: when the sample being tested was a sludge sample from a reactor with CER addition, the first control was a sludge sample with no CER addition and the second control was the CER only mixed at the same extraction conditions without any sludge in it. Time dependent polymer extraction tests were done for 8 hours. Hourly samples were collected from the liquid phase, centrifuged and analyzed for their polymer content. During these tests the amount of CER added to the sludge samples was either 50 or 70 g/gVS. To check the possibility of cell lysis, oxygen uptake rate (OUR) of microorganisms after each extraction was measured. A significant drop in OUR would indicate a significant cell lysis, therefore if such a case happened, the experiments were discontinued to prevent contamination of extracellular polymers with intracellular materials.

Effect of CER dose on the extraction of polymers was tested next. In this part doses applied were 25, 50, 70, 100 and 150 gCER/gVS. The duration of extraction was kept constant at 5 hours. Both effect of time and CER dose were tested on 3 sets of reactors (a total of 6) with 3 different C/N ratios.
Extracellular polymer measurements
Following extractions by CER, the liquid fractions were collected, centrifuged and analyzed for polymer content. Carbohydrate contents of the extracted polymers were measured in duplicate samples by using phenol-sulfuric acid method (Dubois et al. 1956) using alginate as the standard. Protein contents of the polymers were analyzed in duplicate samples by using Bradford (Bradford, 1976) and Lowry (Lowry et al., 1951) methods using BSA as the standard.

Other analytical techniques
TS, MLSS and MLVSS contents of sludges were analyzed using Standard Methods (APHA, 1998). OUR was measured in a sealed flask by a DO probe after washing the sludge samples and resuspending them in PBS as described by Standard Methods (APHA, 1998).

Results and discussion
Development of the CER extraction technique
First the time dependent behavior of the CER extraction technique is investigated. Once the reactors are at steady state washed CER in amounts of either 50 or 70 g/gVS is added to sludge samples taken from the reactors. Table 2 shows the liquid fraction protein and carbohydrate concentrations measured at each hour during the CER extractions for reactors 1 through 4. These results show that both protein by the two methods used and the carbohydrate concentrations increase up to 5 hours of extraction time. After 5 hours there is not a significant change in measured concentrations of protein and carbohydrate. In both of the controls employed along with the samples, carbohydrates and proteins were not detected. The oxygen uptake rate of microorganisms following CER extractions are reported in Table 3.

Evaluation of the results in Tables 2 and 3 together, indicates that prolonged extraction times above 5 hours do not help with the polymer extraction but they negatively affect the OUR of microorganisms. This may be a slight inhibition of microorganisms or some degree of cell lysis. Therefore, the optimal extraction time with CER is selected as 5 hours. Since similar results are obtained in set 1 (reactors 1 and 2) and set 2 (reactors 3 and 4), 8 hour extraction tests were not performed for set 3 (reactors 5 and 6) with the expectation that these reactors would yield similar trends in time dependent experiments.

In the next stage of the study the effect of CER dose on the amount of polymer extracted

Table 2 Time dependent polymer extraction results with the cation exchange resin; to reactors 1 and 2, 50 g CER/gVS, to reactors 3 and 4, 70 g CER/gVS is added

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Reactor 1 (C/N = 17.5)</th>
<th>Reactor 2 (C/N = 17.5)</th>
<th>Reactor 3 (C/N = 5)</th>
<th>Reactor 4 (C/N = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>B 0.0 L 0.0 D 0.0</td>
<td>B 0.0 L 0.0 D 0.0</td>
<td>B 0.0 L 0.0 D 0.0</td>
<td>B 0.0 L 0.0 D 0.0</td>
</tr>
<tr>
<td>1</td>
<td>1.6 5.0 5.4 1.0</td>
<td>1.0 4.6 4.4 1.3</td>
<td>2.3 6.5 2.4 2.4</td>
<td>2.4 6.7 2.2</td>
</tr>
<tr>
<td>2</td>
<td>2.5 14.4 12.0 2.3</td>
<td>2.6 13.1 10.7 3.3</td>
<td>3.3 14.4 2.8 3.4</td>
<td>3.4 15.6 2.7</td>
</tr>
<tr>
<td>3</td>
<td>2.9 15.5 12.3 2.6</td>
<td>2.6 14.6 11.4 5.6</td>
<td>5.6 22.5 3.8 5.6</td>
<td>5.6 23.0 4.4</td>
</tr>
<tr>
<td>4</td>
<td>3.8 19.6 14.8 3.7</td>
<td>3.8 18.3 14.2 6.8</td>
<td>6.8 28.8 5.3 6.8</td>
<td>6.8 28.3 5.3</td>
</tr>
<tr>
<td>5</td>
<td>4.2 18.9 13.8 3.8</td>
<td>3.8 17.8 13.9 6.7</td>
<td>6.7 28.7 6.6 6.8</td>
<td>6.8 29.4 6.8</td>
</tr>
<tr>
<td>6</td>
<td>4.0 19.8 13.9 3.7</td>
<td>3.7 18.1 13.7 6.7</td>
<td>6.7 28.7 6.6 6.7</td>
<td>6.7 29.4 6.8</td>
</tr>
<tr>
<td>7</td>
<td>4.0 19.4 14.2 – – –</td>
<td>6.7 28.5 6.6 6.8</td>
<td>6.8 29.2 6.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.1 19.0 14.5 – – –</td>
<td>6.7 28.6 6.6 6.7</td>
<td>6.7 29.3 6.8</td>
<td></td>
</tr>
</tbody>
</table>

B: Protein by Bradford method (mg/gVS)
L: Protein by Lowry method (mg/gVS)
D: Carbohydrates by Dubois method (mg/gVS)
is investigated. The CER doses applied are 25, 50, 70, 100 and 150 g/gVS. Figure 1 presents the results obtained on the extracted polymer concentration with increasing amount of CER applied for all the reactors operated. In this figure, reactors 1 and 2, reactors 3 and 4 and reactors 5 and 6 are replicate reactors. As can be seen from Figure 1, replicate reactors give very reproducible results. From Figure 1, it is obvious that all polymer concentrations increase with increasing CER concentrations. This increase is sharp up to CER concentration of 70 g/gVS and it slows down after 100 CER/g/gVS. This response is similar for all the reactors. The increase in polymer concentration is negligibly small when CER concentration is increased from 100 to 150 g/gVS except for the carbohydrate data in the last set of reactors (5 and 6). Even for these reactors a decrease in the increase rate of carbohydrates from 70 to 100 and then 150 gCER/gVS is obvious. It can be concluded that the polymers extractable have reached a steady maximum somewhere around 100 to 150 gCER/gVS by this method. Oxygen uptake rate measurements are also conducted on CER extracted sludges and average values for reactors 1 and 2 are reported in Table 4. Similar results were obtained in other reactors.

Examining the results of polymer extraction and OUR data the CER method used in this study has been found to be a suitable method for polymer extraction. The reason for this is that the method extracts significant quantities of polymers from sludge as demonstrated by the results in Figure 1. Besides as seen in Table 3 the method at the end of a 5 hour extraction causes small decreases in OUR during extraction therefore it causes either little or no cell disruption. Minimization of cell lysis is an important consideration in the extraction of extracellular polymers as also noted by many previous studies (Gehr and Henry, 1983; Brown and Lester, 1980). One note about the OUR data is that with an increase of CER to 150 gCER/gVS, the overall decrease in OUR is significant. So a concentration of 100 gCER/gVS is considered as optimal.

Results show that in all the extracts the quantity of protein measured by Lowry and Bradford methods are quite different. Lowry method gives 3 to 5 times higher concentrations. Both of these techniques have been used extensively in measuring sludge extracellular proteins. Frolund et al. (1996) used both methods and in their study also, the Lowry method resulted in higher protein estimations. They found that the Bradford method underestimated the protein concentrations.

### Table 3  
Comparison of OUR of microorganisms during the 50 gCER/gVS extractions (average of reactor 1 and reactor 2 results reported)

<table>
<thead>
<tr>
<th>Time of extraction (hr)</th>
<th>OUR (mg/L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
<td>5.1</td>
</tr>
<tr>
<td>6</td>
<td>4.9</td>
</tr>
<tr>
<td>8</td>
<td>4.6</td>
</tr>
</tbody>
</table>

### Table 4  
OUR for sludges following 5 hr CER extraction

<table>
<thead>
<tr>
<th>CER concentration (g/gVS)</th>
<th>OUR (mg/L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>5.1</td>
</tr>
<tr>
<td>70</td>
<td>4.9</td>
</tr>
<tr>
<td>100</td>
<td>4.6</td>
</tr>
<tr>
<td>150</td>
<td>4.2</td>
</tr>
</tbody>
</table>
This study investigates the effect of three C/N ratios: 5, 17.5 and 40. One main difference in results of these 3 sets of reactors is that their steady state MLSS and MLVSS concentrations are notably different. As the C/N ratios increased, the concentrations of both MLSS and MLVSS increase. MLVSS concentrations increase from 1850, to 3410, and then to 6536 mg/L for C/N ratios of 5, 17.5 and 40, respectively. This is as expected since in high C/N ratio wastewaters, most of the carbon in the system is used in cell synthesis, so these wastes produce high quantities of MLVSS. Parallel to these findings Magesan et al. (2000) state that as the wastewater C/N ratio is increased both microbial biomass and carbohydrate

**Figure 1** Variation of the amount of extracted polymers with the added CER concentration (■ Lowry protein, ◆ Bradford protein, △ Carbohydrate)
content of the cells increase. Also Heldal et al. (1996) notes that for many bacteria at nitrogen limitation conditions with excess carbon sources the cells will accumulate large amounts of various polymers such as carbonaceous glycogen, lipids and phosphates.

Polymers extracted from microorganisms that are cultured at each of these C/N ratios are graphically presented in Figure 1. The calculated average results from replica reactors are presented in Table 5. Assuming the optimum extraction conditions are represented, the results obtained from 100 gCER/gVS extraction are presented in Figure 2. Both Table 5 and Figure 2 demonstrate that as the C/N ratio increases, carbohydrate content of the extracted polymer increases sharply. On the other hand with increasing C/N ratios, the protein content of the extracted polymers decreases. This decrease is obvious with both of the protein measurement techniques even though the absolute values of protein by either technique are quite different.

When the C/N ratio is 5, the amount of nitrogen in the feed is high as compared to carbon. Microorganisms utilize this carbon in the synthesis of proteins and nucleic acids. Since MCRT is 8 days, this nitrogen has a low chance of being used in nitrification. Effluent ammonia nitrogen amounts are low and removal percentages of ammonia nitrogen are 81, 86, 98, for C/N ratios of 5, 17.5 and 40, respectively. For this high amount of nitrogen in the feed almost all carbon is utilized by the microorganisms especially in biomass synthesis rather than in extracellular polysaccharide production and the nitrogen in the system is used in the synthesis of proteins. Since under low carbon conditions microorganisms cannot afford to spend the carbon source in extracellular polysaccharide production, the extracted carbohydrates are low (Table 5 and Figure 2). Rather this carbon is used in energy and synthesis functions. Under these conditions the relatively high concentrations of proteins produced can function in the cell or can exist in the extracellular medium. This is the reason why high concentrations of protein are observed in the extracts at C/N ratio of 5.

### Table 5  Summary of polymers extracted at different C/N ratios with various CER concentrations

<table>
<thead>
<tr>
<th>CER added (g/gVS)</th>
<th>C/N = 5</th>
<th>C/N = 17.5</th>
<th>C/N = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>L</td>
<td>D</td>
</tr>
<tr>
<td>25</td>
<td>4.1</td>
<td>14.6</td>
<td>4.5</td>
</tr>
<tr>
<td>50</td>
<td>5.7</td>
<td>22.8</td>
<td>5.6</td>
</tr>
<tr>
<td>70</td>
<td>6.8</td>
<td>28.7</td>
<td>6.7</td>
</tr>
<tr>
<td>100</td>
<td>8.1</td>
<td>32.0</td>
<td>7.7</td>
</tr>
<tr>
<td>150</td>
<td>8.2</td>
<td>32.6</td>
<td>7.9</td>
</tr>
</tbody>
</table>

B: Protein by Bradford method (mg/gVS)
L: Protein by Lowry method (mg/gVS)
D: Carbohydrates by Dubois method (mg/gVS)

### Figure 2  The variation of polymer concentration with different C/N ratios at 100 gCER/gVS
On the other hand, as the C/N ratio is increased to 17.5 and then to 40, microorganisms can use the excess carbon in the synthesis of cells and also in extracellular polysaccharide production. The carbohydrate concentration in the extracts increases with increasing C/N ratios (Table 5 and Figure 2). Similar results are reported for wastewaters used in land treatment. When the C/N ratio of the wastewater increases, the soil hydraulic conductivity is found to decrease significantly (Magesan et al., 2000). This is explained by the excessive production of carbohydrate containing extracellular polymers at high C/N ratios (C/N = 27:1 and 66:1 as compared to 2.5:1). This finding is in parallel to our findings; at a C/N ratio of 40, excessive production of carbohydrate containing polymers is demonstrated by this work.

In contrast to what Bura et al. (1998) report, results of this study indicate that the protein and carbohydrate fractions are at similar levels for the sludge samples produced at a C/N ratio of 17.5 (typical of activated sludge systems treating municipal wastewaters). Results show that at 100 gCER/gVS extraction, carbohydrate and protein concentrations are 19.1 and 26.5 mg/gVS, respectively. One other important finding of the current study is that the feed composition in terms of carbon and nitrogen content is an important determinant in extracellular polymer composition. This also explains some findings in the literature that report high protein concentrations. Higgins and Novak (1997) report that the bound protein concentration released by the addition of sodium ions is much higher relative to the bound polysaccharide concentration released. These lab-grown microorganisms received high concentrations of total nitrogen even though ammonium ions are low. The COD to total nitrogen content is 6.5 and there is severe carbon restriction in the media. This is comparable to our lowest C/N ratio of 5, with which we have similar observations of the relative abundance of protein and carbohydrate. So with these types of results it would be misinterpretation of the data to just conclude that activated sludge extracellular polymers are higher in protein content rather than in polysaccharide content without considering the C/N ratio of the feed.

Bura et al. (1998) reported that when the molar ratio of C:N:P is decreased from 100:5:1 to 100:1:1, the decrease in protein content is obvious. Our observations are parallel to this; increase of C/N ratio (in terms of COD/NH4.) from 17.5 to 40 causes a marked decrease in polymer protein content. For this condition the current study finds significant increase in the carbohydrate content of the polymer (Figure 2). This result is in parallel to the results of studies by Bengtsson (1991) and Allison and Sutherland (1987) report for pure culture microorganisms. Unfortunately, Bura et al. (1998) reports unchanged levels of carbohydrate polymer with the increase of C/N molar ratio from 20 to 100, which is hard to explain.

In studying the sludge extracellular polymers, the researchers should keep in mind that the extracellular polysaccharides are produced from the cell wall with a specific function, and the other components of the polymer network such as proteins exist there due to processes like cell lysis or enzyme entrapment. Physiology of the microorganisms affects the relative distribution of proteins and carbohydrates. This may better help in understanding the composition and function of the sludge polymer components.

Conclusions

Results of this study show that the microorganism concentration in the reactors is a strong function of C/N ratio. As C/N ratio increases, the MLSS and MLVSS concentrations are observed to increase. C/N ratio also influences the relative distribution of polymer carbohydrate and protein. At low C/N ratio of 5, polymer extracts had high protein and low carbohydrate contents. As the C/N ratio is increased to 17.5 and then to 40, carbohydrate concentration increases sharply and protein concentration decreases. For the C/N ratio typical of activated sludge systems treating municipal wastewaters the protein and carbohydrate contents are comparable.
References
Allison, D.G. and Sutherland, I.W. (1987). The role of exopolysaccharides in adhesion of freshwater bacte-
Health Association, American Water Work Association, Water Environment Federation, Washington,
D.C.
Bradford, M.M. (1976). A rapid and sensitive method for the quantification of microgram quantities of pro-
Appl. Env. Microbial., 40, 179–185.
Composition of extracellular polymeric substances in the activated sludge floc matrix. Wat. Sci. Tech.,
Burdman, S., Jurkevitch, E., Soria-Diaz, M.E., Serrano, A.M.G. and Okon, Y. (2000). Extracellular polysac-
Forster, C.F. and Clarke, A.R. (1983). The production of polymer from activated sludge by ethanolic extrac-
New York, NY, 736 pages.
Harris, R.H. and Mitchell, R. (1973). The role of polymers in microbial aggregation. A. Rev. Microbiol., 27,
27–50.
Horan, N.J. and Eccles, C.R. (1986). Purification and characterization of extracellular polysaccharide from