# METHOD FOR ACCUMULATION OF POLYHYDROXYALKANOATES IN BIOMASS WITH ON-LINE MONITORING FOR FEED RATE CONTROL AND PROCESS TERMINATION

## Abstract of the Invention

A method or process for producing polyhydroxyalkanoates (PHAs) in biomass. The process entails feeding an organic carbon containing substrate to biomass enriched in PHA accumulating bacteria. Particularly the process entails intermittently supplying the substrate to the biomass at least three separate times over a selected period. The object of the process is to produce PHA having a relatively high molecular weight, at least 400,000 g/mole. By controlling the frequency at which the substrate is supplied to the biomass and by feeding a sufficient amount of the substrate to the biomass, the method or process produces the PHA having the relatively high molecular weight.

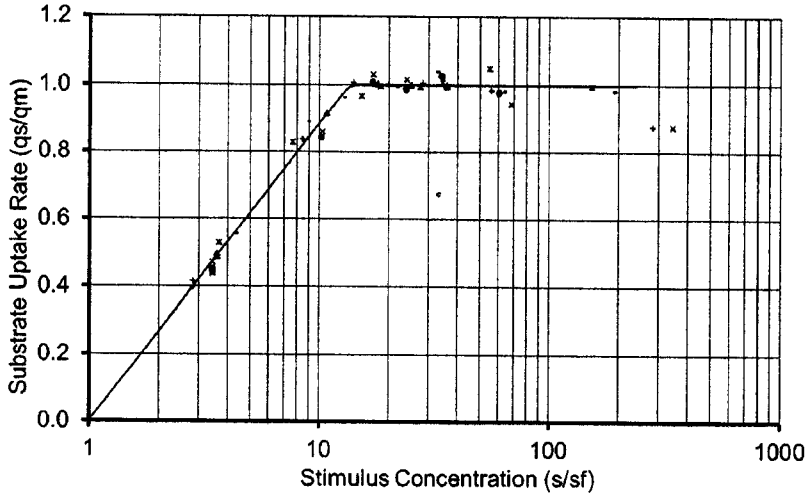


Figure 1

We Claim:

1. A fed-batch method for producing high molecular weight polyhydroxyalkanoates (PHAs) in biomass, comprising:

directing biomass into a reactor having at least one stimulating zone and at least one maintenance zone;

intermittently and repeatedly stimulating the biomass respiration rate during a PHA accumulation process by exposing at least a fraction of biomass in at least one stimulating zone to events of elevated substrate concentration while the biomass as a whole is otherwise maintained with substrate levels that are less than the stimulating substrate concentrations and wherein biomass is circulated and recirculated between the stimulating and maintenance zones;

monitoring and assessing, at least indirectly, the respiration rate of the biomass in the non-stimulating reactor zone(s) where substrate levels are on average less than the stimulating zone substrate concentrations;

controlling the frequency of biomass recirculation or rate of substrate addition such that the respiration rate of the biomass in the maintenance zone(s) does not decrease by more than 70% of the extant achievable maximum respiration rate of the biomass;

retaining the biomass in the reactor until a significant level of PHA is accumulated by the biomass;

harvesting PHA rich biomass from the reactor;

wherein repeated exposures of the biomass or biomass fractions to the elevated stimulating substrate concentrations is from substrate directed to the stimulating zones, such zones containing recirculated fractions of the process total biomass;

wherein the elevated substrate concentrations, achieved by mixing influent substrate with recirculated biomass and used to repeatedly stimulate the biomass respiration rate, is between 10 and 1000 mg-COD/L but preferably between 20 and 500 mg-COD/L;

wherein the above combination of method steps provides for PHA accumulation and wherein the accumulated PHA, when separated from the biomass, includes an average molecular weight of at least 400,000 g/mole.

2. The method of Claim 1 including assessing the biomass respiration rate in the maintenance zone(s) with monitoring methods such as UV/VIS spectroscopy, conductivity, ion electrodes, other chemical sensors, or combinations thereof.

3. The method of Claim 1 wherein the substrate and biomass are mixed together to form a substrate and biomass mixture with elevated substrate concentration, and the method includes controlling the frequency that any given biomass fraction is subject to stimulation by elevated substrate concentration as a function of the concentration of dissolved organic carbon or the concentration of dissolved COD in the maintenance zones.

4. The method of Claim 1 wherein the substrate and biomass are mixed together to form a substrate and biomass mixture with elevated stimulating substrate concentration, and the method further includes controlling the frequency that the biomass or fractions thereof are subject to events of such stimulation by elevated substrate concentration based on the biomass respiration derived from monitoring dissolved oxygen and or carbon dioxide concentrations.

5. The method according to Claim 1 wherein the reactor comprises a batch reactor and at least one separate tank and wherein the biomass is directed into the batch reactor and is contained within mixed liquor in the batch reactor, and the method includes directing the mixed liquor from the batch reactor to at least one separate tank and feeding the substrate to the biomass in the separate tank, and wherein the separate tank includes a volume of mixed liquor less than the volume of mixed liquor in the batch reactor, and wherein the average concentration

of the substrate in the separate tank is higher than the average concentration of the substrate in the batch reactor.

6. The method of Claim 1 wherein controlling the frequency that the biomass or fractions thereof are subject to stimulation by elevated substrate concentration events includes:

(i) intermittently pumping the substrate into a reactor that includes the biomass,

(ii) circulating biomass from one zone having a relatively low average concentration of substrate to a stimulating zone having a relatively high average concentration of substrate wherein the substrate may be fed continuously or non-continuously.

7. The method of Claim 1 wherein the biomass is contained within mixed liquor and the substrate is fed intermittently to the biomass in the stimulating zone; and wherein the frequency of feeding the substrate to the biomass is controlled by sensing the dissolved oxygen concentration in the mixed liquor and feeding substrate to the biomass in response to an increase in dissolved oxygen concentration in the mixed liquor, over a selected time, that is greater than a selected threshold value.

8. The method of Claim 7 including controlling the frequency of feeding the substrate to the biomass by correlating the dissolved oxygen concentration in the mixed liquor with biomass respiration rate, and feeding of the substrate to the biomass in response to a decrease in respiration rate, over the selected time, that is less than a selected threshold value.

9. The method of Claim 1 including treating a wastewater stream and producing a mixed cultured biomass and harvesting the mixed cultured biomass and feeding the mixed cultured biomass to a batch reactor; and

wherein the method entails utilizing at least a portion of the wastewater stream or an alternative wastewater stream as a source for the substrate fed to the mixed cultured biomass.

10. The method according to any of Claims 1, wherein the point from which to begin harvesting PHA-rich biomass or to terminate the fed-batch process of PHA accumulation is determined by turbidometric measurements based on near-infrared back scatter methods, Raman infrared spectroscopy, build-up of chemical oxygen demand (RBCOD), decreased substrate uptake rates relative to the substrate used, decreased biomass respiration rate relative to the substrate used, or a combination thereof.

Dated this the 12th Day of June, 2012

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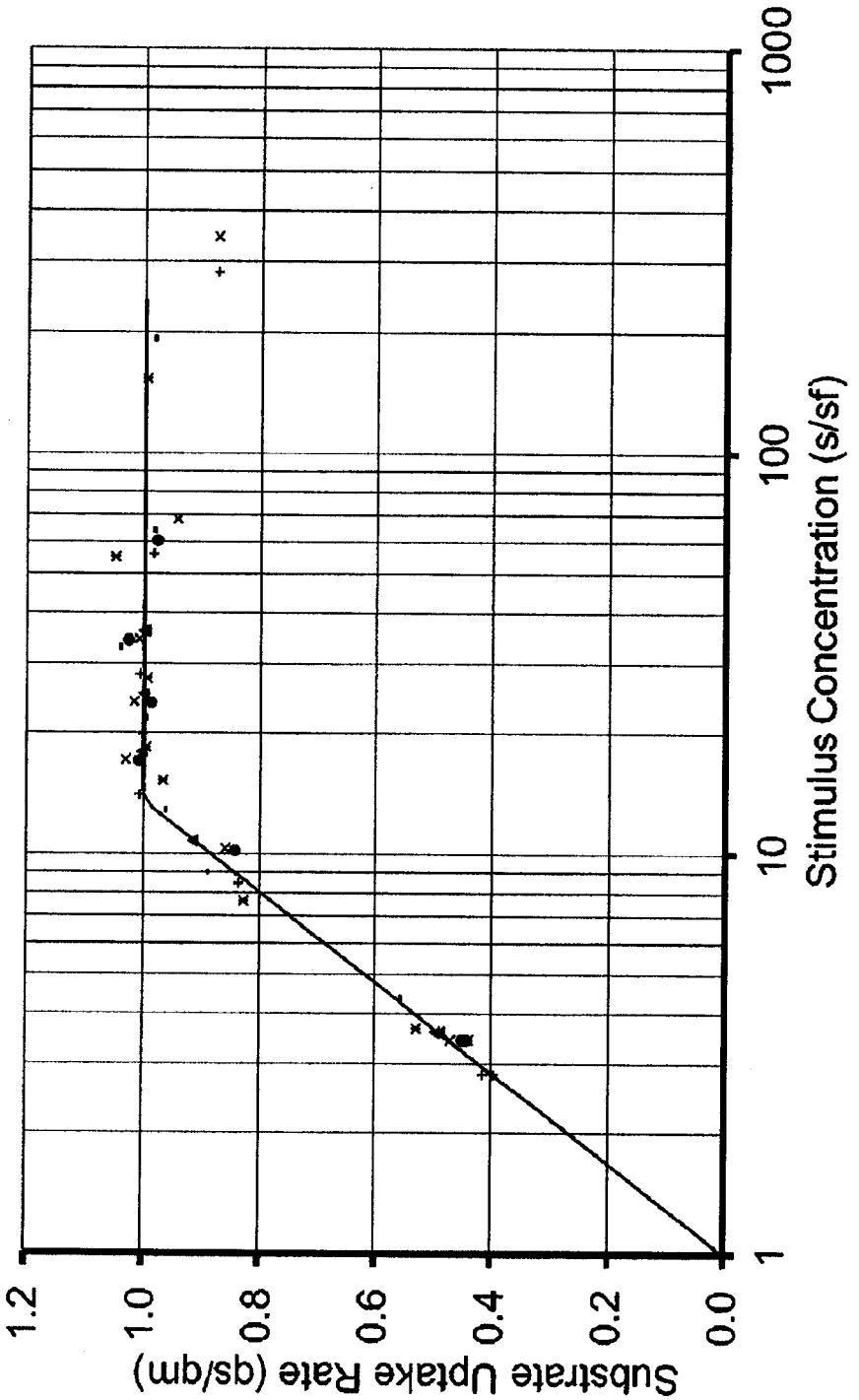


Figure 1



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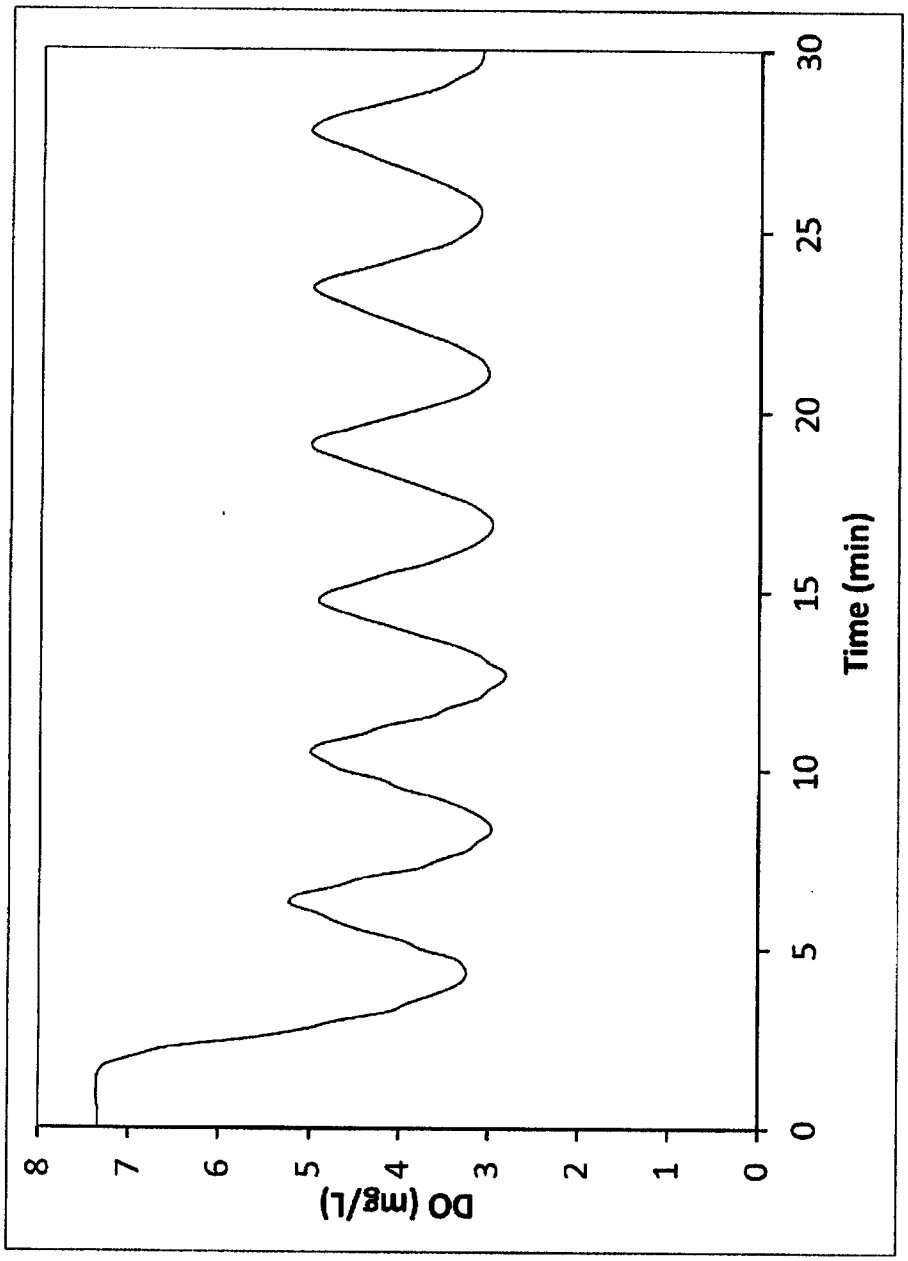


Figure 2



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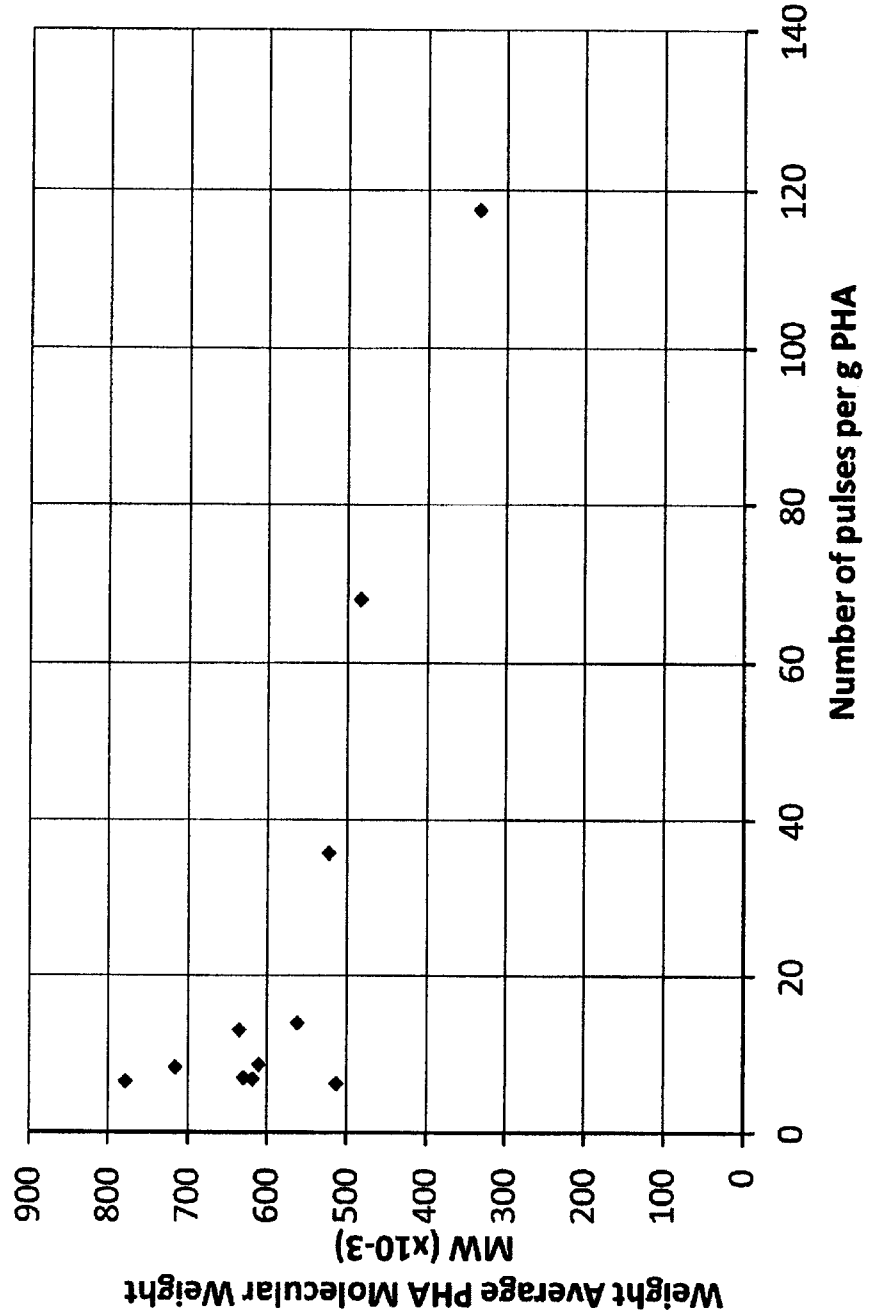


Figure 3

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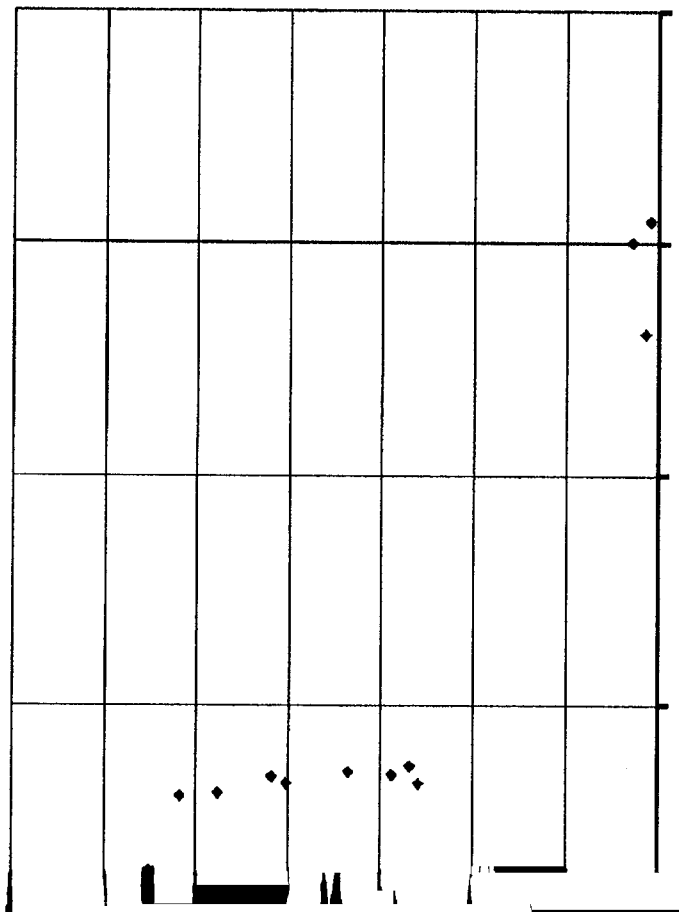


Figure 4

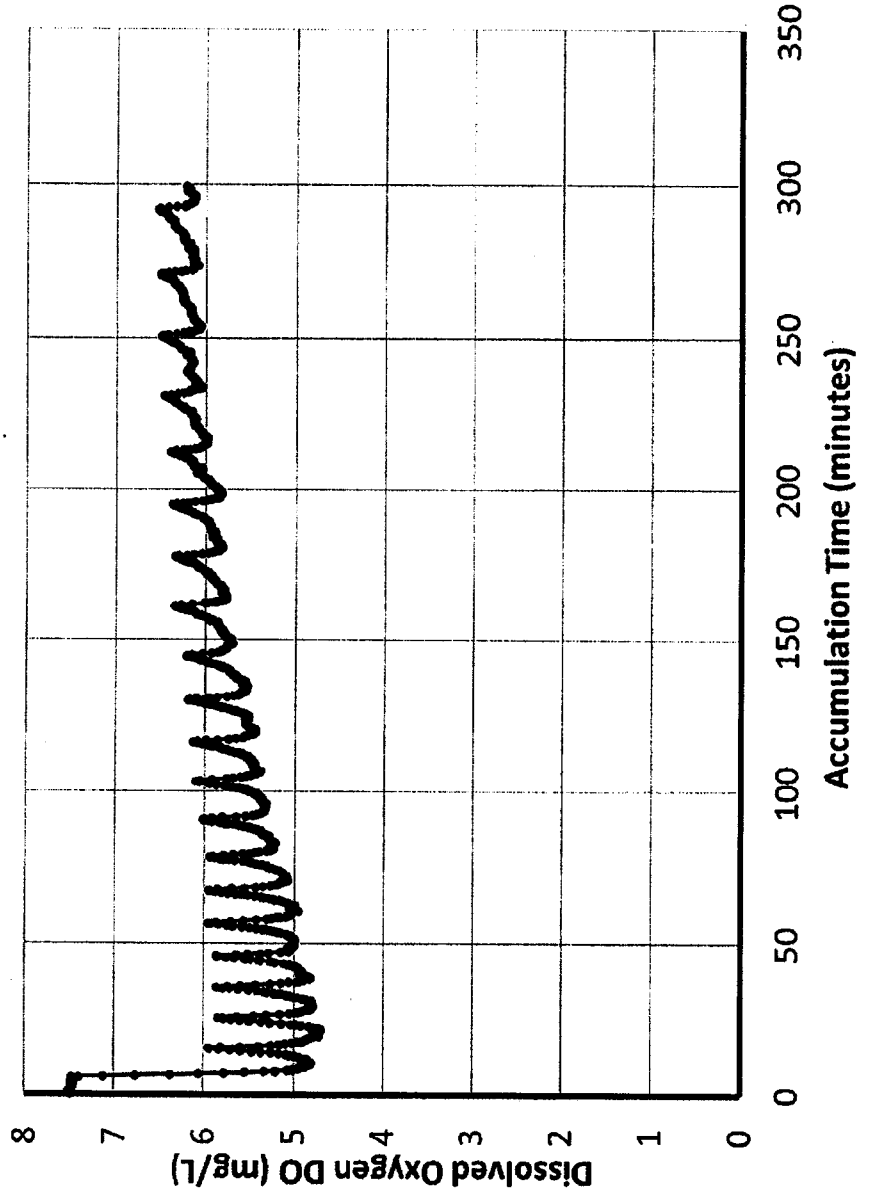


Figure 5

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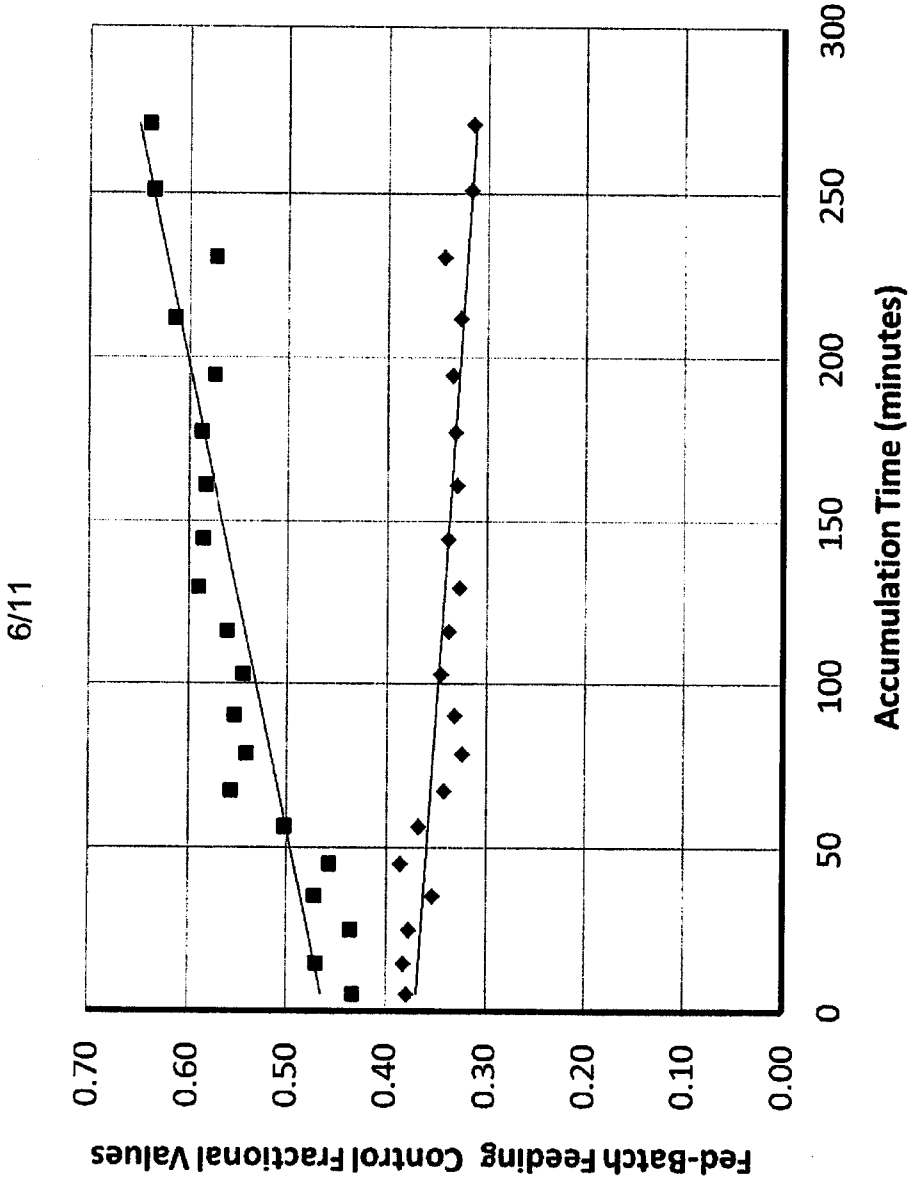


Figure 6

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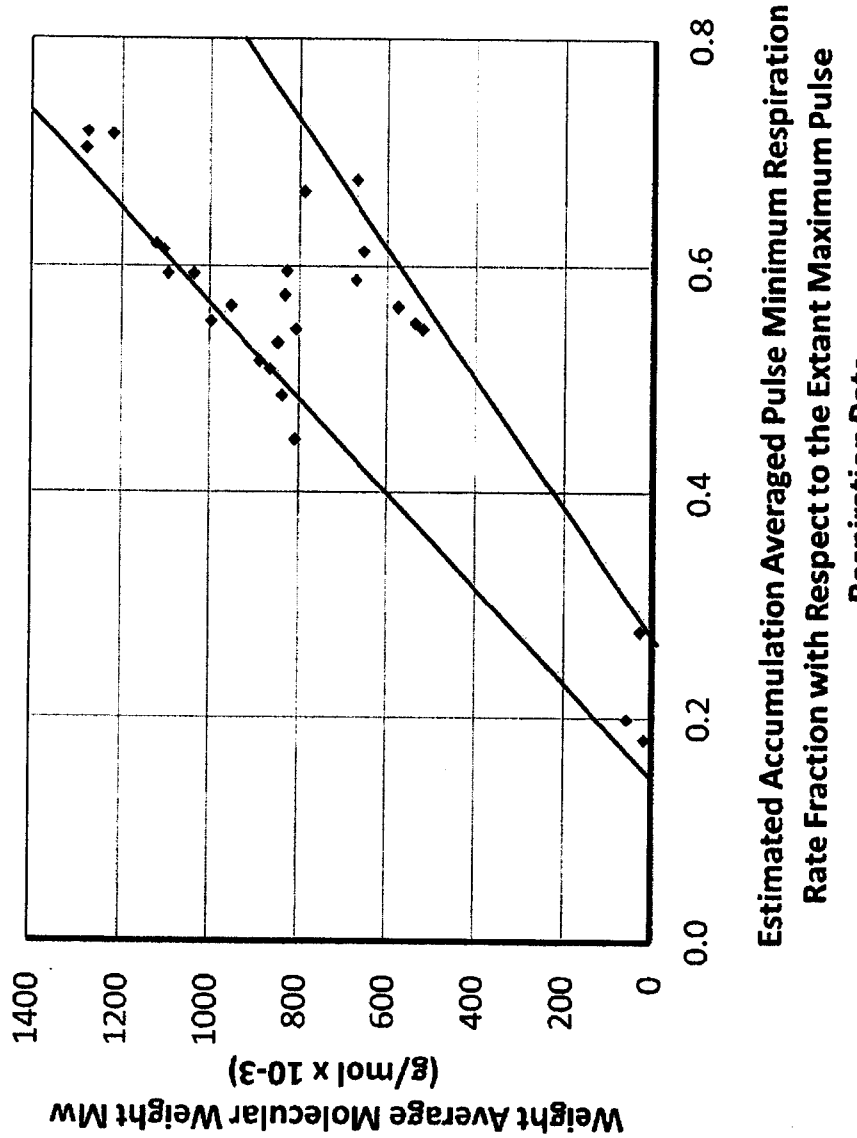


Figure 7

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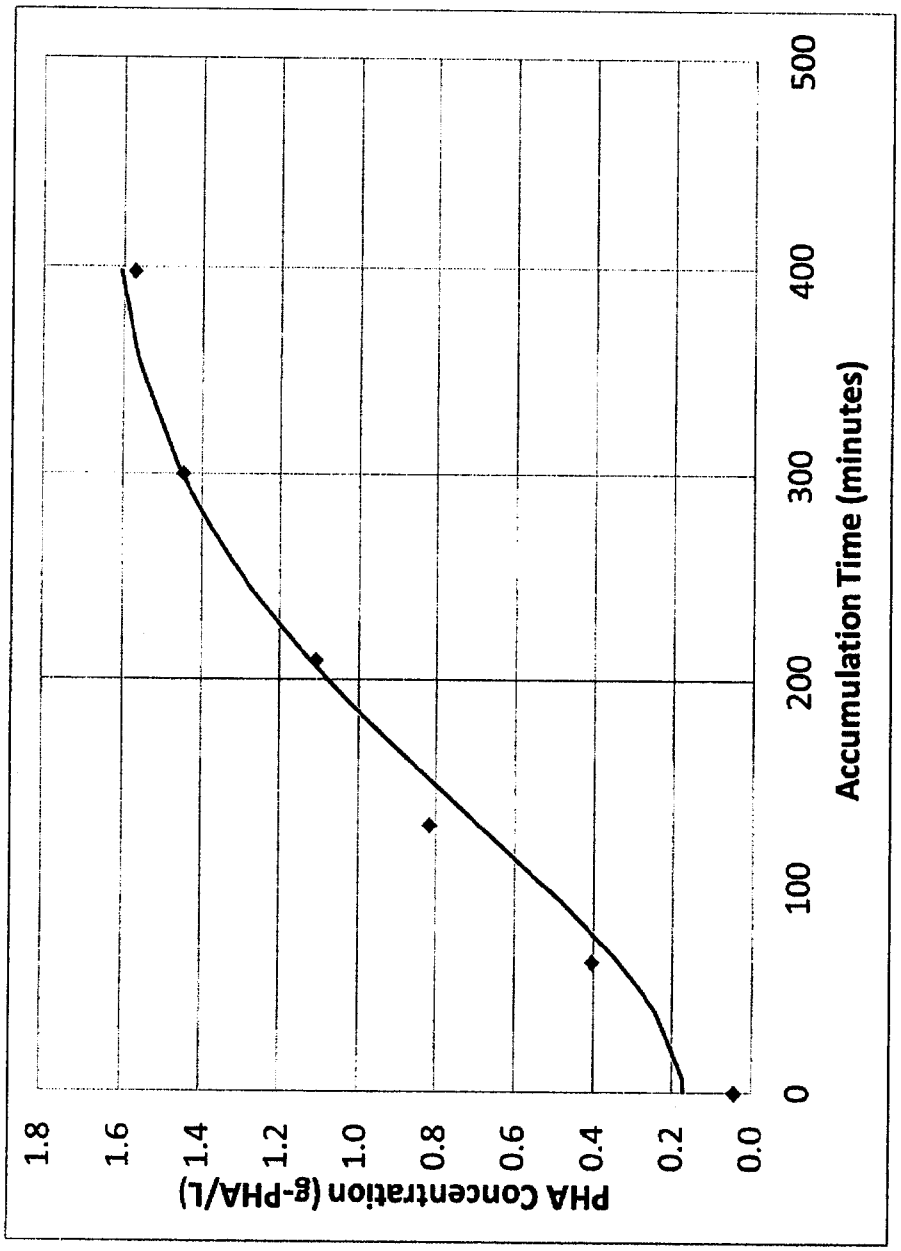


Figure 8



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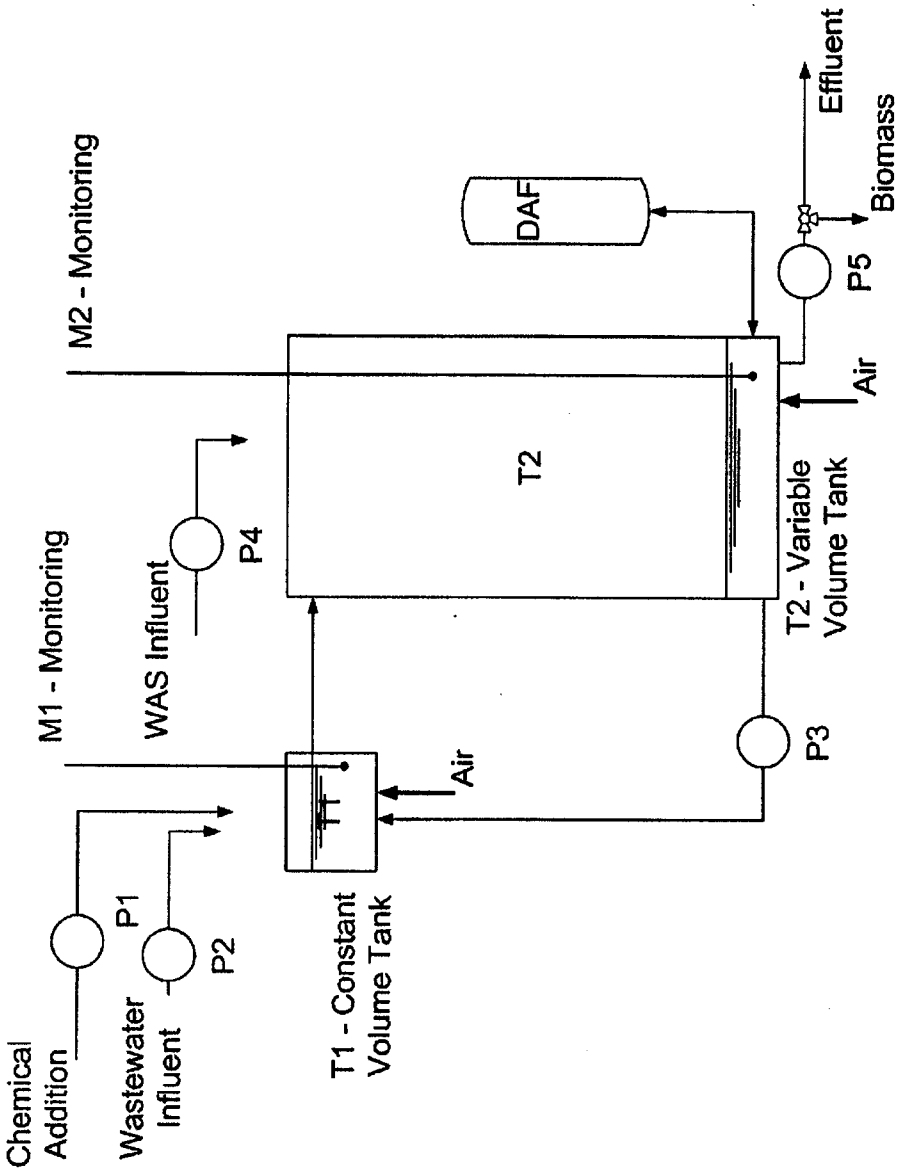


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Figure 9

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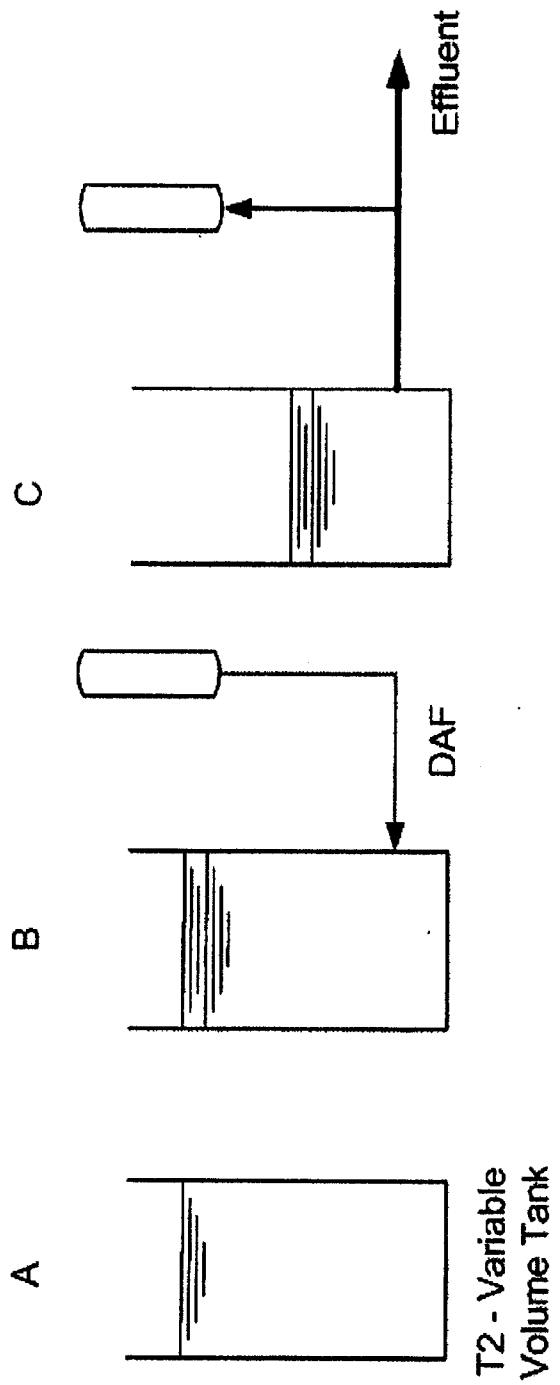


Figure 10

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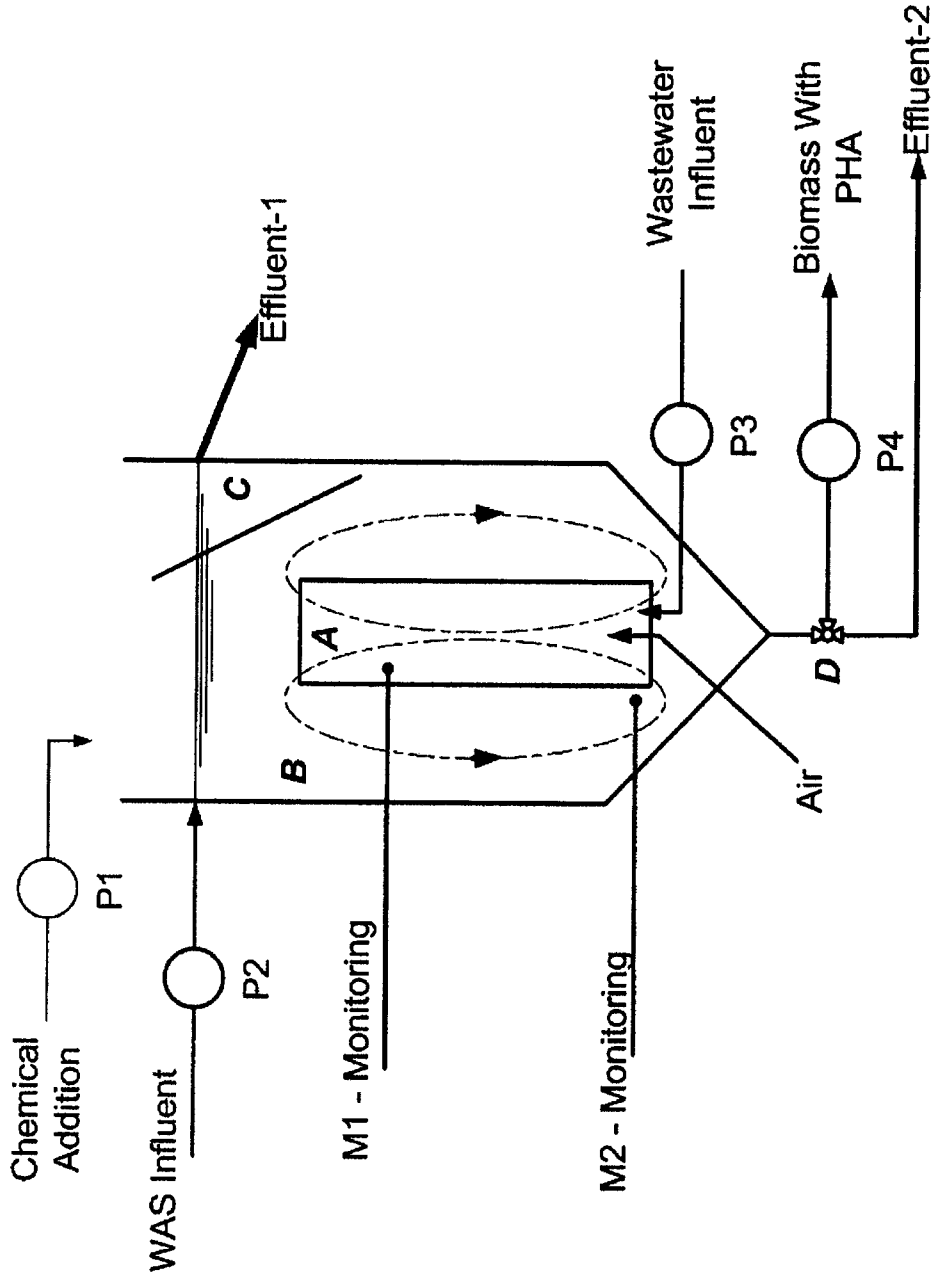


Figure 11

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